



Original Article

Gamma-irradiation and synthesized silver nanoparticles from neem seeds stimulate leaf morphometric traits and nutritional profiles of Pigeon pea [*Cajanus cajan* (L.) Millsp]

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ABSTRACT

This study investigated the combined influence of gamma irradiation and silver nanoparticles (AgNPs) on the proximate composition and mineral profile of the leaves of White and Red Testa pigeon pea (*Cajanus cajan* L.), with the aim of enhancing its nutritional quality. Pigeon pea seeds were exposed to gamma radiation at doses of 0, 100, 200, and 400 Gray (Gy) using a Cobalt-60 source and subsequently primed with green-synthesized AgNP solutions at concentrations of 0, 25, 50, and 100 ppm. The experiment was arranged in a factorial design, yielding 16 treatment combinations, including an untreated control (0 Gy + 0 ppm AgNPs). Results showed that the interaction between gamma irradiation and AgNPs produced distinct dose-dependent effects. In the Red Testa pigeon pea, moderate radiation levels (100–200 Gy) combined with 25–50 ppm AgNPs significantly enhanced key agronomic performance such as leaf morphometry and nutritional profiles. Proximate analysis revealed that crude protein increased at low irradiation doses, peaking at 8.98% (R3:400 Gy + 25 ppm AgNPs), while carbohydrate and energy contents rose markedly under higher combined treatments, reaching 34.60% and 194.71 Kcal/100 g, respectively relative to the control. Mineral composition was also improved at moderate treatments, with enhanced bioavailability of potassium, calcium, magnesium, and phosphorus. Similar trends were also observed in the White Testa Pigeon Pea; moderate combinations—particularly 100 Gy + 50 ppm (W4) and 200 Gy + 100 ppm (W8)—dry matter, protein, and carbohydrate contents, as well as essential minerals such as potassium, magnesium, and phosphorus. Optimal results were achieved between 100–200 Gy and 50–100 ppm AgNPs, suggesting that this integrated approach can be used to improve pigeon pea breeding pipeline in Nigeria.

Keywords: Nutritional quality, Leaves, White Testa, Red Testa, Pigeon Pea, Leaf Area, Breeding

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INTRODUCTION

Legumes occupy a central place in the diets of many populations, particularly in

developing countries where access to animal proteins is limited. They play a major role in alleviating protein–energy

malnutrition by providing an affordable and sustainable source of plant-based proteins, essential amino acids, minerals, and vitamins (1). Edible legumes such as beans, lentils, cowpeas, and pigeon peas are integral to food security and nutrition due to their rich nutrient profile and adaptability to diverse agro-ecological conditions. However, despite their nutritional value, the production and utilization of legumes have not kept pace with the increasing protein demand of the rapidly expanding global population. This shortfall is further exacerbated by factors such as declining soil fertility, land scarcity, and the dominance of cereal-based diets that are typically lower in protein and certain micronutrients (2).

In response to this nutritional gap, researchers have advocated for the exploration and utilization of underexploited legumes as potential alternatives to conventional protein sources (3). Among these, pigeon pea (*Cajanus cajan*), a drought-tolerant and versatile legume, has shown significant promise. It is an important grain legume cultivated in tropical and subtropical regions of the world, notable for its resilience under harsh environmental conditions such as low rainfall, poor soil fertility, and high temperatures (4). Pigeon pea is culturally and economically important in many developing regions, including Nigeria, where it is known by various local names: "Fio-fio" among the Igbo, "Otili" among the Yoruba, and "Waken Kurawa" among the Hausa (5). It is known for its multipurpose utility: the grains serve as a source of food for humans, the leaves and husks are used as animal feed, and the stems provide firewood and fencing material for rural households (6). Agriculturally, pigeon pea contributes to soil fertility through its

ability to fix atmospheric nitrogen via symbiotic association with *Rhizobium* bacteria, thereby reducing dependence on chemical fertilizers (7).

The perennial nature of pigeon pea plant allows it to produce tender leaves shortly after cutting the plants during its vegetative growth period and after the harvest of seed crop. The fodder yields of pure stand cuts depend on both genotype and management practices, which include height and frequency of cuttings, availability of soil moisture and nutrition. Leaves are the major forage component during vegetative growth. As the plant approaches reproductive stage, the fodder quality is enhanced due to the development of high-protein seeds. Therefore, the forage quality at a particular time will depend on the proportion of different plant parts. It is unclear how gamma radiation affects the proteins, vitamins, carbohydrates, and minerals in pigeon peas, and different gamma radiation dosages may have different impacts on the nutritional value of the food. Closing this gap is essential to determining if gamma radiation is suitable for pigeon pea preservation while retaining its nutritional content (8).

Among the emerging technologies to address these challenges, gamma irradiation has gained recognition as an innovative and environmentally safe approach for improving food quality and safety. Gamma irradiation, a form of ionizing radiation, involves exposing food materials to controlled doses of gamma rays, typically emitted from isotopes such as cobalt-60 or cesium-137 to induce beneficial molecular and biochemical changes (9). This technique has been widely used in food science for purposes such as preservation, microbial

decontamination, pest control, and enhancement of functional and nutritional properties.

Ultimately, it is unclear how gamma radiation affects the proteins, vitamins, carbohydrates, and minerals in pigeon peas, and different gamma radiation dosages may have different impacts on the nutritional value of the food. It has also not been documented, the synergistic effect of gamma irradiation and synthesized silver nanoparticles on the nutritional compositions of pigeon pea. Closing this gap, it is essential to determine if gamma radiation and synthesized silver nanoparticles are suitable for pigeon pea improvement without compromising its nutritional compositions.

MATERIALS AND METHODS

The Red and White Testa pigeon pea seeds used for this study, with the accession numbers NGB05525 and NGB05543 respectively, were obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria. The seeds were authenticated at the institute before use in the experiment.

Gamma irradiation was conducted using a Cirrus Cobalt-60 (^{60}Co) at Teletherapy unit at the Radiology and Oncology Department, Ahmadu Bello University Teaching Hospital, Shika, Zaria. The Atomtex device is a self-calibrated, leakage-free radiation dosimeter designed to measure dose rates within an operating range of 50 nSv to 10 Sv/h. The Cobalt-60 source had a radioactivity strength of 229.061 TBq (6190.84 Ci) at the time of treatment.

The experiment was laid out in a factorial design with two factors: Factor A: Gamma irradiation doses (4 levels: 0, 100, 200, and

400 Gy) and Factor B: Silver nanoparticle concentrations (4 levels: 0, 25, 50, and 100 ppm). This resulted in 16 treatment combinations, with the control group (0 Gy + 0 ppm AgNPs) and nine specific treatment combinations focusing on irradiated seeds with AgNP treatments

Seed Preparation for Irradiation

Dried pigeon pea seeds were sorted to remove damaged or discoloured seeds. Selected seeds were packaged in appropriate containers suitable for gamma irradiation treatment.

Biosynthesis of Silver Nanoparticles

Aqueous solution 1 mM of silver nitrate (AgNO_3) was prepared in 250 ml Erlenmeyer flask, and 10 % leaf extract was added for reduction of Ag^+ ions. The complete mixture was kept on magnetic stirrer at 30 °C. Time and colour change will be recorded along with periodic sampling and scanning by UV-Visible (UV-Vis) spectrophotometer. Suitable controls were maintained all through the conditions of experiments. The Complete reduction of Ag^+ ions was confirmed by the change in colour from light or faint to yellowish colloidal brown. The colloidal solution was then kept aside for 24 hours for complete bio-reduction and saturation denoted by UV-Vis spectrophotometric scanning. The solution was sealed and stored properly for further use. The formation of silver nanoparticles will be further confirmed by different spectrophotometric analysis (10).

Seed Treatment

The biosynthesized silver nanoparticles were dissolved at different concentrations (0, 25, 50, 75 and 100 ppm) in de-ionized water. Irradiated pigeon pea seeds were

subjected to priming by soaking in the silver nanoparticles solution for about 2 hours. The treated seeds were then dried under shade before planting (11) containers until sowing.

Field Experimental Design

The experiment was laid in a randomized complete block design (RCBD); seeds of two (2) genotypes were sown alongside with their respective controls. Two seeds were sown per hole; and then thinned to one after five weeks. The inter and intra row spacing of 40 × 25 cm were used.

Treatment Groups

The gamma-irradiated seeds were divided into nine treatment groups based on the combination of gamma irradiation doses and AgNP concentrations. For the Red Testa Pigeon Pea, the following treatment combinations were used: R1: 100 Gy + 25 ppm AgNPs; R2: 200 Gy + 25 ppm AgNPs; R3: 400 Gy + 25 ppm AgNPs; R4: 100 Gy + 50 ppm AgNPs; R5: 200 Gy + 50 ppm AgNPs; R6: 400 Gy + 50 ppm AgNPs; R7: 100 Gy + 100 ppm AgNPs; R8: 200 Gy + 100 ppm AgNPs; and R9: 400 Gy + 100 ppm AgNPs. For the White Testa Pigeon Pea, the following treatment combinations were used: W1: 100 Gy + 25 ppm AgNPs; W2: 200 Gy + 25 ppm AgNPs; W3: 400 Gy

+ 25 ppm AgNPs; W4: 100 Gy + 50 ppm AgNPs; W5: 200 Gy + 50 ppm AgNPs; W6: 400 Gy + 50 ppm AgNPs; W7: 100 Gy + 100 ppm AgNPs; W8: 200 Gy + 100 ppm AgNPs and W9: 400 Gy + 100 ppm AgNPs.

RESULTS

The results showed that there exists variation in the responses of both pigeon pea genotypes. The weight of leaves of the Red Testa pigeon pea was moderately influenced by 400 Gy + 25 ppm AgNPs (R3), producing the highest weight of leaves (9.65 g); this value was significantly different ($p < 0.05$) from R9 (400 Gy + 100 ppm AgNPs) with a value of 7.24 g (Table 1). Meanwhile, in the White Testa pigeon pea, the highest leaf weight (8.70 g) was produced by W4 (100 Gy + 50 ppm AgNPs) which was not significantly different ($p > 0.05$) from all the other values (Table 1). For the leaf area in the Red Test Pigeon Pea, R5 (200 Gy + 50 ppm AgNPs) had significantly highest (35.15 cm²) leaf area when compared with the lowest (12.33 cm²) due to R3. W4 (100 Gy + 50 ppm AgNPs) produced significantly highest leaf area (38.84 cm²) in the White Testa pigeon pea (Table 1). Similar trends were also observed for both leaf length and leaf breath in the two genotypes.

Table 1: Effect of Gamma Irradiation and Silver Nanoparticles on Leaf Morphometric Traits of Pigeon Pea

Treatment	Fresh Weight of Leaves (g)	Length of Leaf (cm)	Breadth of Leaf (cm)	Area of Leaf (cm ²)
RED TESTA PIGEON PEA				
R3	9.65 ± 1.10 ^b	4.78 ± 1.12 ^a	3.62 ± 0.18 ^a	12.88 ± 2.80 ^a
R4	8.23 ± 0.49 ^{ab}	10.84 ± 1.00 ^{bc}	3.96 ± 0.36 ^a	33.26 ± 5.86 ^b
R5	8.41 ± 0.58 ^{ab}	11.58 ± 0.95 ^c	3.98 ± 0.21 ^a	35.13 ± 4.26 ^b
R7	7.68 ± 0.45 ^a	10.42 ± 0.74 ^{bc}	3.96 ± 0.28 ^a	31.48 ± 4.42 ^b
R8	8.05 ± 0.25 ^{ab}	10.18 ± 0.30 ^{bc}	3.94 ± 0.18 ^a	30.24 ± 2.31 ^b
R9	7.24 ± 0.90 ^a	9.14 ± 0.72 ^{ab}	3.28 ± 0.26 ^a	23.03 ± 3.54 ^{ab}
R Control	7.81 ± 0.36 ^{ab}	10.02 ± 0.46 ^{bc}	3.76 ± 0.20 ^a	28.52 ± 2.61 ^b
WHITE TESTA PIGEON PEA				
W1	6.80 ± 0.56 ^a	9.16 ± 0.73 ^a	4.28 ± 0.24 ^{ab}	29.86 ± 0.77 ^a
W2	8.50 ± 1.61 ^a	10.24 ± 0.08 ^{bc}	3.98 ± 0.05 ^a	30.56 ± 0.41 ^a
W3	7.51 ± 1.11 ^a	10.70 ± 0.08 ^{bc}	4.58 ± 0.12 ^b	36.78 ± 1.21 ^b
W4	8.70 ± 0.37 ^a	11.30 ± 0.20 ^c	4.58 ± 0.09 ^b	38.84 ± 1.25 ^b
W7	8.00 ± 0.39 ^a	10.30 ± 0.25 ^{bc}	4.34 ± 0.14 ^{ab}	33.62 ± 1.80 ^{ab}
W8	6.55 ± 1.17 ^a	10.62 ± 0.24 ^{bc}	4.48 ± 0.09 ^b	35.66 ± 0.85 ^b
W9	7.30 ± 1.15 ^a	9.98 ± 0.30 ^{ab}	4.40 ± 0.18 ^{ab}	33.09 ± 2.30 ^{ab}
W Control	8.43 ± 0.40 ^a	10.54 ± 0.20 ^{bc}	4.42 ± 0.09 ^b	34.90 ± 0.52 ^{ab}

Values are means ± standard error of means. Values followed by the same letter(s) along the column are not significantly different at $p < 0.05$ as tested by DMRT.

Effect of Gamma Irradiation and Silver Nanoparticles on the Proximate Composition of Pigeon Pea Leaves

In the Red Testa pigeon pea, the proximate composition of pigeon pea leaves following combined gamma and AgNP treatments showed some interesting variations. Dry matter ranged between 34.01% (R7) and 49.35% (R9), while moisture content varied inversely (35.60–65.99%). Ash content ranged from 1.48 % (R5) to 2.40% (R4). Crude fat decreased steadily with increasing treatment intensity, from 7.62% (Control) to 2.04% (R9). Crude protein ranged between 4.47% (Control) and 8.98% (R3), with R3 recording the highest protein level. Carbohydrate content increased from 15.93% (R3) to 34.60% (R9), while energy

values peaked at 194.71 Kcal /100 g (R9). The results of the proximate composition of white testa pigeon pea seeds subjected to different combinations of gamma irradiation and silver nanoparticles are presented in Table 2. Moisture content ranged from 64.64% to 70.16%, with the highest value (70.16%) observed in W2 (200 Gy + 25 ppm) and the lowest (64.64%) in W9 (400 Gy + 100 ppm). The dry matter content showed an inverse relationship with moisture content, ranging from 29.84% to 35.36%. The highest dry matter (35.36%) was recorded in W9, followed closely by W8 (35.32%) and W4 (35.30%), indicating enhanced solid biomass accumulation under these treatments. Ash content ranged from 0.74% to 2.95%, with the maximum value (2.95%) observed in the control, while W4

(1.98%) recorded the highest among treated samples, suggesting moderate mineral retention. Crude fat content varied significantly ($p < 0.05$) among treatments, ranging between 2.01% and 10.63%. The highest crude fat (10.63%) was recorded in the control, followed by W8 (8.26%), while the least (2.01%) occurred in W2 (200 Gy + 25 ppm). Crude protein content ranged from 4.73% to 7.27%, with the highest value (7.27%) obtained in W1 (100 Gy + 25 ppm), which was significantly different ($p < 0.05$) from the other treatments. Moderate protein levels were observed in W4 (6.00%), W7 (6.26%), and W9 (6.57%), while W3 (400 Gy + 25 ppm) had the least protein value (4.73%). Crude fibre content ranged between 0.92% and 3.69%, with the highest (3.69%) found in the control, and the lowest ($0.92 \pm 0.32\%$) in W9 (400 Gy + 100 ppm). Carbohydrate (CHO) content

varied from 10.37% to 22.07%, with W7 (100 Gy + 100 ppm) having the highest (22.07%) and the control recording the lowest (10.37%).

Energy values ranged from 115.97 to 173.51 kcal/100 g, with W8 (200 Gy + 100 ppm) exhibiting the highest energy value (173.51 kcal/100 g), followed by W9 (166.62 kcal/100 g). The lowest energy value (115.97 kcal/100 g) was observed in W2 (200 Gy + 25 ppm); this highest value was significantly different from all the other treatments. Overall, treatments involving moderate gamma irradiation (100–200 Gy) combined with higher nanoparticle concentrations (50–100 ppm) tended to enhance the dry matter, fat, and energy content of the seeds, while excessive irradiation (400 Gy) resulted in a reduction of some proximate constituents

Table 2: Effect of Gamma Irradiation and Silver Nanoparticles on the Proximate Composition of Leaves of Red and White Testa Pigeon Pea

Treatment	Dry Matter (%)	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Protein (%)	Crude Fibre (%)	CHO (%)	Energy (Kcal / 100 g)
RED TESTA PIGEON PEA								
R3	34.77 ± 0.00 ^b	65.41 ± 0.17 ^b	1.73 ± 0.24 ^{bc}	5.96 ± 0.00 ^d	8.98 ± 0.31 ^d	2.51 ± 0.05 ^c	15.93 ± 0.23 ^b	153.30 ± 2.16 ^d
R4	34.98 ± 0.01 ^b	65.02 ± 0.01 ^b	2.40 ± 0.07 ^d	5.28 ± 0.00 ^c	7.09 ± 0.44 ^{bc}	2.99 ± 0.09 ^d	17.22 ± 0.29 ^{bc}	144.74 ± 0.57 ^b
R5	35.21 ± 0.00 ^b	64.79 ± 0.00 ^b	1.48 ± 0.00 ^{ab}	3.99 ± 0.00 ^b	7.31 ± 0.04 ^{bc}	1.85 ± 0.00 ^b	20.59 ± 0.04 ^c	147.49 ± 0.01 ^b
R7	34.01 ± 0.01 ^a	65.99 ± 0.01 ^b	2.17 ± 0.19 ^{cd}	4.38 ± 0.05 ^{bc}	8.36 ± 0.04 ^{cd}	2.71 ± 0.24 ^c	16.39 ± 0.52 ^b	138.42 ± 1.47 ^a
R8	35.19 ± 0.00 ^b	64.80 ± 0.00 ^b	2.20 ± 0.25 ^{cd}	3.31 ± 0.06 ^{ab}	5.95 ± 0.09 ^{ab}	2.75 ± 0.31 ^c	20.97 ± 0.70 ^c	137.50 ± 1.94 ^a
R9	49.35 ± 0.65 ^c	35.60 ± 1.05 ^a	1.99 ± 0.21 ^c	2.04 ± 0.06 ^a	8.23 ± 0.09 ^{cd}	2.49 ± 0.03 ^c	34.60 ± 0.84 ^d	194.71 ± 2.51 ^e
R Control	34.43 ± 0.01 ^{ab}	65.57 ± 0.01 ^b	1.97 ± 0.00 ^c	7.62 ± 0.00 ^e	4.47 ± 0.00 ^a	2.46 ± 0.01 ^c	17.91 ± 0.01 ^b	158.09 ± 0.08 ^c
WHITE TESTA PIGEON PEA								
W1	35.09 ± 0.01 ^e	64.91 ± 0.01 ^a	0.97 ± 0.01 ^a	5.30 ± 0.01 ^d	7.27 ± 0.09 ^b	1.22 ± 0.02 ^a	20.33 ± 0.13 ^c	158.12 ± 0.12 ^e
W2	29.84 ± 0.16 ^a	70.16 ± 0.16 ^b	1.49 ± 0.00 ^b	2.01 ± 0.34 ^a	6.26 ± 0.66 ^{ab}	1.87 ± 0.00 ^b	18.22 ± 1.16 ^b	115.97 ± 1.05 ^a
W3	31.95 ± 0.05 ^b	68.05 ± 0.05 ^a	1.47 ± 0.00 ^b	4.33 ± 0.00 ^{bc}	4.73 ± 0.26 ^a	1.84 ± 0.00 ^b	19.59 ± 0.31 ^{bc}	136.23 ± 0.20 ^b
W4	35.30 ± 0.03 ^e	64.70 ± 0.03 ^a	1.98 ± 0.01 ^c	3.66 ± 0.01 ^b	6.00 ± 0.04 ^{ab}	2.48 ± 0.01 ^c	21.19 ± 0.09 ^c	141.69 ± 0.25 ^{bc}
W7	34.98 ± 0.00 ^d	65.02 ± 0.00 ^a	1.48 ± 0.00 ^b	3.32 ± 0.02 ^b	6.26 ± 0.13 ^{ab}	1.86 ± 0.00 ^b	22.07 ± 0.15 ^c	143.15 ± 0.06 ^c
W8	35.32 ± 0.01 ^e	64.68 ± 0.01 ^a	1.01 ± 0.02 ^a	8.26 ± 0.00 ^e	4.73 ± 0.09 ^a	1.26 ± 0.02 ^a	20.07 ± 0.12 ^{bc}	173.51 ± 0.09 ^f
W9	35.36 ± 0.05 ^e	64.64 ± 0.05 ^a	0.74 ± 0.25 ^a	6.36 ± 0.01 ^{de}	6.57 ± 1.31 ^b	0.92 ± 0.32 ^a	20.77 ± 0.71 ^{bc}	166.62 ± 2.51 ^e
W Control	34.11 ± 0.00 ^{cd}	65.89 ± 0.00 ^a	2.95 ± 0.01 ^d	10.63 ± 0.30 ^f	6.48 ± 0.09 ^b	3.69 ± 0.01 ^d	10.37 ± 0.23 ^a	163.02 ± 1.44 ^{de}

Values are means ± standard error of means. Values followed by the same letter(s) along the column are not significantly different at $p < 0.05$ as tested by DMRT.

Effect of Gamma Irradiation and Silver Nanoparticles on the Mineral Composition of Pigeon Pea Leaves

The mineral composition of the White Testa pigeon pea under combined treatments is shown in Table 3. Potassium content ranged from 0.169% (Control) to 0.299% (R8: 200 Gy + 100 ppm AgNPs). Sodium content increased with treatment intensity, peaking at 0.078% (R5: 200 Gy + 50 ppm AgNPs). Calcium concentration was highest in R9: 400 Gy + 100 ppm AgNPs (0.423%), while magnesium ranged from 0.014% (R3) to 0.093% (R4). Phosphorus content varied between 0.009% (R5) and 0.106% (R7), showing increased bioavailability at moderate treatment levels. The results of the mineral composition of white testa pigeon pea leaves subjected to various combinations of gamma irradiation and silver nanoparticles are presented in Table 3. Potassium content ranged from 0.199% to 0.295%, with the highest value ($0.295 \pm 0.005\%$) observed in W7 (100 Gy + 100 ppm AgNPs) and the lowest ($0.199 \pm 0.002\%$) in W9 (400 Gy + 100 ppm). This indicates that moderate doses of gamma irradiation coupled with higher nanoparticle concentrations enhanced potassium accumulation in the seeds.

Sodium content varied between 0.037% and 0.052%. The highest sodium level (0.052%) was recorded in W1 (100 Gy + 25 ppm), which was not significantly different ($p > 0.05$) from W2 (200 Gy + 25 ppm) and the control (0.051%). The lowest sodium content (0.037%) occurred in W9 (400 Gy + 100 ppm), suggesting that higher irradiation doses reduced sodium accumulation. Calcium content ranged from 0.174% to 0.338%, with the maximum (0.338%) observed in control,

followed by W3 (0.325%) and W1 (0.324%). The lowest calcium level (0.174%) was recorded in W2 (200 Gy + 25 ppm), which was significantly different ($p < 0.05$) from the control and other treatments. Magnesium content showed significant variation ($p < 0.05$) across treatments, ranging from 0.017% to 0.107%. The highest magnesium concentration (0.107%) occurred in W9 (400 Gy + 100 ppm), while the least (0.0173%) was recorded in W3 (400 Gy + 25 ppm). This suggests that elevated radiation levels may promote magnesium uptake when combined with high nanoparticle concentrations. Phosphorus content varied between 0.053% and 0.105%, with the highest (0.105%) observed in W9 (400 Gy + 100 ppm), which was significantly different ($p < 0.05$) from the control (0.092%) and all other treatments. The lowest phosphorus value (0.053%) was recorded in W3 (400 Gy + 25 ppm).

Overall, treatments involving moderate gamma doses (100–200 Gy) combined with nanoparticle levels between 50–100 ppm generally enhanced the accumulation of essential minerals such as potassium and phosphorus. However, excessive radiation (400 Gy) tended to reduce calcium and sodium concentrations, indicating possible radiation-induced disruption in mineral metabolism.

Table 3: Effect of Gamma Irradiation and Silver Nanoparticles on Mineral Composition of Leaves of Red and White Testa Pigeon Pea

Treatment	Potassium (%)	Sodium (%)	Calcium (%)	Magnesium (%)	Phosphorus (%)
RED TESTA PIGEON PEA					
R3	0.292 ± 0.002 ^c	0.070 ± 0.001 ^e	0.332 ± 0.000 ^d	0.014 ± 0.002 ^a	0.095 ± 0.001 ^d
R4	0.176 ± 0.001 ^a	0.036 ± 0.000 ^a	0.146 ± 0.120 ^a	0.093 ± 0.001 ^f	0.087 ± 0.00 ^c
R5	0.280 ± 0.001 ^d	0.078 ± 0.001 ^d	0.272 ± 0.002 ^b	0.082 ± 0.001 ^e	0.009 ± 0.000 ^a
R7	0.280 ± 0.001 ^d	0.077 ± 0.000 ^d	0.321 ± 0.001 ^c	0.056 ± 0.001 ^d	0.106 ± 0.001 ^f
R8	0.299 ± 0.001 ^c	0.051 ± 0.001 ^c	0.275 ± 0.001 ^b	0.056 ± 0.001 ^d	0.089 ± 0.000 ^c
R9	0.190 ± 0.001 ^b	0.048 ± 0.001 ^b	0.423 ± 0.001 ^e	0.051 ± 0.001 ^c	0.101 ± 0.001 ^{ef}
Control	0.169 ± 0.001 ^a	0.034 ± 0.000 ^a	0.273 ± 0.001 ^b	0.049 ± 0.003 ^b	0.052 ± 0.000 ^b
WHITE TESTA PIGEON PEA					
W1	0.251 ± 0.001 ^e	0.052 ± 0.001 ^e	0.324 ± 0.002 ^e	0.021 ± 0.003 ^a	0.095 ± 0.000 ^f
W2	0.274 ± 0.001 ^f	0.051 ± 0.001 ^{de}	0.174 ± 0.002 ^a	0.070 ± 0.002 ^e	0.063 ± 0.000 ^b
W3	0.238 ± 0.000 ^c	0.040 ± 0.000 ^b	0.325 ± 0.001 ^e	0.017 ± 0.003 ^a	0.053 ± 0.001 ^a
W4	0.231 ± 0.001 ^c	0.040 ± 0.001 ^b	0.262 ± 0.002 ^c	0.042 ± 0.002 ^c	0.066 ± 0.001 ^{bc}
W7	0.295 ± 0.005 ^g	0.047 ± 0.001 ^c	0.262 ± 0.002 ^c	0.076 ± 0.004 ^d	0.068 ± 0.000 ^{bc}
W8	0.230 ± 0.001 ^c	0.049 ± 0.000 ^{cd}	0.211 ± 0.001 ^b	0.021 ± 0.001 ^a	0.068 ± 0.000 ^{bc}
W9	0.199 ± 0.002 ^a	0.037 ± 0.000 ^a	0.238 ± 0.002 ^{bc}	0.107 ± 0.001 ^e	0.105 ± 0.000 ^e
Control	0.240 ± 0.001 ^{cd}	0.051 ± 0.001 ^{de}	0.338 ± 0.002 ^f	0.032 ± 0.000 ^b	0.092 ± 0.000 ^d

Values are means ± standard error of means. Values followed by the same letter(s) along the column are not significantly different at $p < 0.05$ as tested by DMRT.

DISCUSSION

The combined exposure of pigeon pea seeds to gamma irradiation and silver nanoparticles (AgNPs) produced distinct leaf morphometric and certain biochemical responses across the treatment combinations (R1–R9 and W1–W9). The data revealed that moderate irradiation doses (100–200 Gy) combined with low-to-medium AgNP concentrations (25–50 ppm) generally enhanced vegetative growth traits such as leaf area, stem diameter, and plant height. In contrast, higher doses (400 Gy) and elevated AgNP levels (100 ppm) tended to suppress these parameters. Occasionally, the 400 Gy when combined low doses of AgNPs stimulated positive responses. These observations reflect the concept of radiation hormesis, wherein low to moderate ionizing radiation levels stimulate physiological activity, while excessive exposure causes oxidative stress and tissue injury (12).

Enhanced vegetative performance in treatments R4 and R5 (100–200 Gy + 50 ppm AgNPs) suggests a synergistic effect between moderate gamma exposure and nanoparticle-induced stress moderation. AgNPs are known to improve photosynthetic pigment stability, chloroplast integrity, and gas-exchange efficiency, thereby supporting better carbon assimilation under mild radiation stress (13). Concurrently, gamma irradiation at moderate intensities can increase endogenous auxin and cytokinin synthesis, accelerating cell division and elongation. The interaction of these physiological processes likely produced the higher leaf area and stem diameter observed in the moderate treatments. At the higher radiation level of 400 Gy (R3, R8, R9), growth inhibition became apparent, particularly when combined with higher nanoparticle concentrations. This outcome aligns with reports that excessive gamma exposure disrupts membrane integrity, inhibits enzyme

systems, and generates reactive oxygen species that exceed the antioxidant buffering capacity of plant tissues. High AgNP concentrations may also aggravate oxidative imbalance by interfering with ion transport and cellular redox homeostasis. The significant correlations observed among leaf breadth, area, and stem diameter confirm that morphological improvements in optimal treatments were physiologically coordinated, while negative correlations with tree height indicate potential trade-offs between vertical elongation and radial expansion.

Proximate composition data further supported the presence of dose-dependent interactions. Moderate irradiation and nanoparticle combinations improved crude protein and carbohydrate contents, suggesting an adaptive metabolic reorganization under controlled oxidative stress. Such treatments may enhance nitrogen assimilation and protein biosynthesis through activation of key enzymes like nitrate reductase, while AgNPs can stimulate amino-acid and carbohydrate metabolism (14). Conversely, the highest radiation and nanoparticle levels led to elevated dry-matter content but reduced crude fat, reflecting a diversion of carbon skeletons toward carbohydrate synthesis to sustain energy requirements under stress. The decline in lipid content at higher doses may result from peroxidation of membrane lipids caused by excessive free-radical generation.

These biochemical shifts correspond with earlier observations that low-dose gamma irradiation enhances legume nutritional quality and storage potential, whereas excessive exposure disrupts macromolecular stability and digestibility (15). AgNPs appear to buffer these

negative effects by reinforcing antioxidant enzyme activity and improving nutrient utilization efficiency. The increased energy and carbohydrate values recorded for R9 indicate a compensatory response, wherein carbohydrate metabolism intensifies to counteract protein degradation induced by radiation stress.

Mineral analysis also revealed a pattern of moderate enhancement followed by decline at extreme doses. Potassium, sodium, and calcium contents were generally improved under 100–200 Gy combined with 25–50 ppm AgNPs, compared with the control. This improvement could be due to increased membrane permeability and ion transport capacity triggered by mild radiation-induced membrane remodeling and nanoparticle interaction with root uptake systems (16). In contrast, the reduced mineral content at higher doses may result from oxidative damage to root membranes and transport proteins, limiting nutrient uptake efficiency. Phosphorus enrichment in moderate treatments suggests stimulated ATP production and enhanced metabolic energy flow, contributing to better growth and grain development.

The observed variations in morphological traits among the treatments indicate that both gamma irradiation and silver nanoparticles (AgNPs) significantly influenced the growth responses of the White Testa pigeon pea. Moderate combinations, such as 100 Gy + 50 ppm (W4) and 200 Gy + 100 ppm (W8), enhanced leaf area, diameter, and plant height, reflecting improved photosynthetic capacity and resource allocation. These effects also align with the radiation hormesis principle, which postulates that low-to-moderate doses of ionizing radiation stimulate physiological functions

through enhanced enzymatic activation and cellular repair processes (12,13). The positive responses in W4 and W7 further suggest a synergistic interaction between AgNPs and gamma irradiation. AgNPs are known to promote seedling vigor, improve nutrient absorption, and enhance stress tolerance via activation of antioxidant enzymes (15). Their nanoscale size enables efficient penetration into plant tissues, facilitating metabolic enhancement and ion exchange. Conversely, the reduced performance in W3 (400 Gy + 25 ppm) and W9 (400 Gy + 100 ppm) may indicate oxidative damage, DNA degradation, and impaired chloroplast structure resulting from excessive radiation exposure.

In terms of proximate composition, the increase in crude protein and ash under moderate gamma × AgNP treatments reflects enhanced nitrogen assimilation and metabolic activation. Low-dose irradiation can increase enzymatic synthesis and nutrient translocation, while AgNPs may stabilize protein structures and boost amino acid biosynthesis. Increased energy values in W8 and W9 suggest that certain combinations promote carbohydrate accumulation, possibly due to increased photosynthate retention under sublethal stress levels. Mineral element enhancement, particularly potassium and phosphorus under moderate treatments, signifies improved ionic transport and metabolic coordination. Potassium regulates osmotic balance and stomatal conductance, while phosphorus supports nucleic acid synthesis. Overall, the results confirm that low-to-moderate gamma irradiation in combination with AgNPs exerts a hormetic effect, improving morphological development, proximate composition, and mineral nutrient balance, whereas higher

doses induce oxidative stress, metabolic imbalance, and potential toxicity. These findings are consistent with prior reports that emphasize the dual role of ionizing radiation and nanoparticles as both stimulants and stressors depending on concentration and exposure intensity (12,13).

CONCLUSION

The present study investigated the combined effects of gamma irradiation and silver nanoparticles (AgNPs) on the leaf morphometric, proximate composition, and mineral profile of pigeon pea (*Cajanus cajan* L.). The results demonstrated that the interaction between moderate gamma doses (100–200 Gy) and low-to-medium AgNP concentrations (25–50 ppm) significantly improved leaf morphometric and nutritional compositions of pigeon pea relative to the control and high-dose treatments. Moderate combinations (particularly R4 and R5: 100 Gy + 50 ppm and 200 Gy + 50 ppm, respectively) enhanced vegetative growth characteristics such as leaf area, stem diameter, and plant height, as well as improved biochemical composition—particularly crude protein, carbohydrate, and mineral contents.

Treatments W4 (100 Gy + 50 ppm) and W8 (200 Gy + 100 ppm) produced particularly positive outcomes, improving leaf morphology, dry matter accumulation, and overall nutrient content. These enhancements reflect the synergistic action of ionizing radiation and nanomaterials, which can stimulate plant metabolism, improve enzymatic activity, and facilitate better nutrient uptake. Overall, the study concludes that moderate gamma irradiation (100–200 Gy) combined with 50–100 ppm AgNPs yields

the most favorable physiological and biochemical responses in pigeon pea. Therefore, the interactive gamma-AgNP approach represents a promising bio-stimulatory strategy for improving crop quality and productivity, provided that treatment levels are carefully optimized to balance stimulation and stress.

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REFERENCES

1. Kuraz-Abebe, B. (2022). The dietary use of pigeon pea for human and animal diets. *The Scientific World Journal*, 2022(1), 4873008.
2. Marsono, Y., A'yuni, N. R. L., Marseno, D. W., & Triwitono, P. (2022). Physical characteristics, nutrients, and antinutrients composition of pigeon pea (*Cajanus cajan* (L.) Millsp.) grown in Indonesia. *Food Research* 6 (2), 53 - 63.
3. Bhat, R., & Sridhar, K. R. (2009). Influence of electron beam irradiation on the antinutritional features and protein digestibility of seeds of nutraceutically valued legume mucuna pruriens. *Food Processing: Methods, Techniques and Trends*, 1(1), 197-209.
4. Makelo, M. N. (2011). *Pigeon pea production manual for sub-Saharan Africa*. ICRISAT Publication. www.icrisat.com.
5. Esan, V. I., & Ojemola, O. I. (2018). Evaluation of production systems, traditional knowledge of pigeon pea (*Cajanus cajan*) and risks of extinction of pigeon pea, jack bean (*Canavalia ensiformis*) and Lobia Bean (*Lablab purpureus*) in some parts of south west Nigeria. *Journal of Experimental Agriculture International*, 2 (7), 1-11
6. Nwosu, J. N., Ojukwu, M., Ogueke, C. C., Ahaotu, I., & Owuamanam, C. I. (2013). The Anti-nutritional Properties and Ease of De-hulling on the Proximate Composition of Pigeon pea (*Cajanus cajan*) as Affected by Malting. *International Journal of Life Sciences*, 2(2), 60-67.
7. Ayenan, M. A. T., Danquah, A., Ahoton, L. E., & Ofori, K. (2017). Utilization and farmers' knowledge on pigeon pea diversity in Benin, West Africa. *Journal of Ethnobiology and Ethnomedicine*, 13(1), 1-14.
8. Gwoda, C.L.L., Saxena, K.B., Upadhyaya, H.D., & Slim, S.N. (2011) Pigeon pea: Form an orphan to leader in food legumes. *In Biodiversity in Agriculture: Domestication, Evolution and Sustainability* (Gepts P, Bettinger RL, Brush SB, Famula TR, McGuire PE, Qualast CO and Damania AB eds.). Pp361-373.
9. Akaagerger, N. B., & Tsavnande, L. S. (2024). Effect of Gamma Irradiation on Nutritional Quality and Shelf Life of Cowpea (*Vigna unguiculata*) Produced in Benue State Nigeria. *Nigerian Journal of Physics*, 33(3), 10-13.
10. Niharika, N., Shikha, J., Pranav, K., and Vijay, D. (2016). Synthesis and

- characterization of silver nanoparticles via green route. *Korean Journal of Chemical Engineering*, 33 (10), 2990-2997.
11. Khalaki, M.A., Ghorbani, A. and Moameri, M. (2016). Effects of silica and silver nanoparticles on seed germination traits of *Thymus Kotschyanus* in laboratory conditions. *Journal of Rangeland Sciences*, 6(3), 221-230.
 12. Al-Huqail, A. A., & Al-Ghamdi, A. A. (2021). Radiation hormesis effects in plants exposed to low-dose gamma irradiation. *Plant Physiology Reports*, 26(1), 143–152.
 13. Hussain, A., Ali, S., & Ahmed, M. (2022). Silver nanoparticles improve photosynthetic performance under radiation stress. *Journal of Nanobiotechnology*, 20(1), 112.
 14. Ahmed, S., Khan, M., & Rahman, A. (2022). Role of silver nanoparticles in nitrogen metabolism and protein biosynthesis. *Plant Biotechnology Reports*, 16(2), 225–234.
 15. Kumar, R., Sharma, P., & Chauhan, G. (2020). Influence of gamma irradiation on nutritional quality and storage stability of legumes. *Food Chemistry*, 311, 125–135.
 16. Duhan, J. S., Singh, A., & Kumar, P. (2020). Dose-dependent effects of gamma irradiation on mineral composition of pulses. *Radiation Physics and Chemistry*, 170, 108642.