Advances in Research

Volume 27, Issue 3, Page 43-51, 2026; Article no.AIR.157732
ISSN: 2348-0394, NLM ID: 101666096

Effect of Cow-based Organic Inputs and Plant Growth Regulators on Seed Germination and Seedling Growth of Karonda under Protected Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/air/2026/v27i31633

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/157732

Original Research Article

Received: 10/03/2026
Published: 06/05/2026

Abstract

The use of organic waste in proper concentration with scarification may regulate growth behaviour in many fruit crops and pre- sowing treatments of organic waste could lead to increase seed germination and enhancement of seedling growth. The present investigation was conducted to study the effect of pre-sowing seed treatments on germination and growth of karonda (*Carissa carandas L.*) under polyhouse conditions. Different treatments comprising GA_3, cow urine and cow dung were evaluated to determine their influence on germination and seedling development. The results revealed that seed treatments significantly affected all the

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Cite as: *Kumar, L., Suman, M., Kumar, M., & Kaur, G. (2026). Effect of Cow-based Organic Inputs and Plant Growth Regulators on Seed Germination and Seedling Growth of Karonda under Protected Conditions. Advances in Research, 27(3), 43–51. https://doi.org/10.9734/air/2026/v27i31633*

parameters studied. Among the treatments, GA_3 @ 200 ppm for 20 hours (T_9) proved to be the most effective. It recorded the earliest initiation of germination (7.89 days) and the highest germination percentage (78.00%). Although it took relatively more time to complete germination, it resulted in uniform and healthy seedlings. Growth parameters such as plant height (10.19 cm), shoot diameter (3.21 mm), number of leaves (21.39) and fresh and dry weight of leaves (1.24 g and 0.57 g, respectively) were also highest under T_9 treatment, indicating superior seedling vigour. The control (untreated seeds) showed the poorest performance in all aspects, while organic treatments exhibited moderate improvement but remained inferior to GA_3 treatments. Thus, it can be concluded that pre-sowing treatment of karonda seeds with GA_3 @ 200 ppm for 20 hours is highly beneficial for enhancing germination, growth, and overall seedling quality, and is recommended for nursery raising under protected conditions.

Keywords: GA_3; germination; seed treatment; Karonda; seedling growth.

1. Introduction

Karonda (*Carissa carandas* L.) is native to India and grows wild in Maharashtra, Rajasthan, Uttar Pradesh, West Bengal, Madhya Pradesh, Bihar and Andhra Pradesh in wild form (Mahajan *et al.*, 2022; Mishra *et al.*, 2024). Mount Abu region of Rajasthan and Rajgarh region of Madhya Pradesh have its natural variability. It is popularly known as "Bengal currant" or "Christ's Thorn". It belongs to family Apocynaceae with chromosome number 2n = 22. Karonda is an important minor indigenous under exploited fruit crop of India (Meena *et al., 2023*; Sarkar, 2024). It has recently attained importance as an arid zone horticulture crop because of its hardy nature and its nutritious fruits. In India, the mature fruit is harvested for Indian pickles. It contains pectin and accordingly is a useful ingredient in chutney. Ripe fruits exude a white latex when severed from the branch. The unripe fruits of karonda are medicinally used as an astringent. The ripe fruit is sweet, cooling, appeaser and antiscorbutic and is useful in controlling burning sensation, skin diseases, scabies, pruritus and particularly suitable for tarts and puddings. Fruits are generally harvested at immature stage for vegetable purpose, while fully ripen fruits are consumed fresh or processed (Malik *et al.*, 2010). Karonda fruit is a rich source of iron and dried fruits (39.10mg/ 100g) of iron and contains a fair amount of vitamin C. Therefore, it is very useful for cure of anemia and has antiscorbutic properties.

Many horticultural crops including karonda produce recalcitrant seeds which lose their viability when dried below the threshold value. Most recalcitrant seed cannot to gather tolerate moisture below 25 percent and some species are also sensitive to chilling temperature. Karonda is commonly propagated by seed and fresh seeds are sown for raising seedlings in the month of August and September. Pre-sowing treatments with cow urine, cow dung and plant growth regulator like Gibberellic Acid (GA_3) etc. have a significant role on the germination percentage, emergence, seedling height, number of leaves and roots in karonda and other crops. Soaking seeds in aqueous solutions of cow urine and plant growth regulators for (GA_3) 12, 24 and 36 hours has been found to induce early germination, enhance germination percentage and promote seedling growth in fruit crops like Mango, custard apple, citrus, karonda etc.

The use of organic waste in proper concentration with scarification may regulate growth behaviour in many fruit crops and pre- sowing treatments of organic waste could lead to increase seed germination and enhancement of seedling growth. Cow urine contains Iron, urea, uric acid, estrogen and progesterone which affect the inhibitory response to seed germination, shoot growth and seedling vigour. Gibberellic acid (GA_3)is used for weakening of the seed coat so that the radicle of the seedling can break through the seed coat. Gibberellins also help in enhancing the availability of reserved mineral elements which promote the germination process.

2. Materials and Methods

2.1 Location

Kota district is located at 25.18° N to 75.83° E Latitude in South Eastern Rajasthan. It covers an area of 221.36 km^2. Agro-climatically, the district falls in Zone V, known as Humid South Eastern Plain. The average rainfall in the region is 660.6. mm. Maximum temperature range in the summer is 40 to 48°C and minimum 1.0- 2.6°C during winter.

2.2 Treatment Details

The experiment consisted of ten treatments arranged sequentially to evaluate the effects of organic inputs and plant growth regulators under different durations. The experiment was laid out in Completely Randomised Design (CRD) and replicated thrice. The treatments were as follows: T_1 (Control), where no treatment was applied; T_2, cow urine at 30% concentration for 8 hours; T_3, cow urine at 30% concentration for 12 hours; T_4, cow urine at 30% concentration for 16 hours; T_5, cow urine at 30% concentration for 24 hours; T_6, cow dung treatment for 10 hours; T_7, cow dung treatment for 20 hours; T_8, GA_3 at 200 ppm for 10 hours; T_9, GA_3 at 200 ppm for 20 hours; and T_{10}, GA_3 at 100 ppm for 24 hours. These treatments were applied in a systematic sequence to compare the influence of varying durations and concentrations of organic amendments and growth regulators on the growth and development of the crop.

2.3 Preparation of Nursery

The site selected for nursery was a fine and well drained soil. It was close to the source of irrigation. The soil was dug upto a depth of 20 cm and thoroughly pulverized. Well rotten FYM. and leaves mould in equal quantities were added evenly in the soil. The soil of the nursery was sterilized with a mixture of formation and water prepared in ratio of 1:100. Five liter of this mixture was applied to the 900 square cm of the soil surface, to prevent any possible infection of the seeds by fungal and insect pest. The treated soil was covered with polythene for 2 days. The seed bed was raised about at least to cm above ground level. The surface of the bed was made fine and smooth by using a wooden plank.

2.4 Experimental Technique

In all the studies the material comprised of Karonda seed collected from well ripened fruits. Mature and well ripened fruits were taken for extraction of seed. After separation of seed from fruits were washed with fresh water and kept in shade. Roughly and unwanted seed were neglected and freshly extracted seed were used for sowing in the well prepared and raised nursery seed beds.

2.5 Preparations of Growth Regulator Solutions

The stock solution (100 ppm) of GA_3 was prepared by dissolving 0.1 gm of GA_3 in 10 ml of methanol and after that making a volume of one liter by adding pure distilled water. Methanol which is essential for preparing an accurate and homogeneous stock solution. At such low concentrations, methanol evaporates quickly and does not have any toxic or inhibitory effect on seed germination or seedling growth.

2.6 Method of Seed Treatment

After solution of cow urine, cow dung and growth regulators were transferred separately into different beaker. The cow dung was used as well decomposed slurry. The counted lot of 300 seed were soaked in the respective growth regulators solution and control in distilled water for 24 hours. After soaking the seed, seed were spread in the shade. The seeds were dried for 10 minutes in shade after soaking. The dried seeds were immediately sown in the nursery beds which were previously watered. In control, the seeds were sown in the nursery beds with water soaking. Seed were slightly covered with thin layer of fine sieved manure and soil.

2.7 Sowing of Seeds

Treated seed were sown on 24th September, 2025 in well prepared nursery in randomized sub plot. Sowing of seed was done by hand dibbling method with 5 cm deep at the distance of 30 cm plant to plant and 45 cm row to row. After sowing the seeds were covered with the mixture of sand and well rotten F.Y.M. light irrigation was given after sowing the seed, later on irrigation was given from time to time as and when needed.

2.8 Observations Recorded

The present investigation included detailed observations on germination, shoot growth, and root development parameters under different treatments. For germination studies, seeds were monitored daily to record the number

of days taken for the initiation of germination in each treatment. The number of days required to attain 50 percent germination was calculated by counting days from the onset of germination until half of the total seeds had germinated. Germination percentage was determined by counting the total number of germinated seeds in each treatment and expressing it as a percentage of the total number of seeds sown using the standard formula: Germination (%) = (Number of germinated seeds / Total number of seeds sown) × 100.

Shoot observations were recorded at regular intervals. The length of seedlings (plant height) was measured at 30, 60, and 90 days after sowing (DAS) using a meter scale, from ground level to the tip of the fully opened leaf. The diameter of seedlings was measured just above the ground surface with the help of a vernier caliper at the same intervals. The number of leaves per plant was counted from randomly selected seedlings at 15-day intervals. Fresh weight of leaves was recorded by immediately weighing freshly harvested leaves from selected plants using an electronic weighing balance, and the average was calculated. For dry weight, the leaves were oven-dried at 60°C for 12 hours to remove moisture and then weighed to obtain dry weight per plant. Seedling Vigour Index (SVI) was calculated by multiplying germination percentage with root length. Leaf area was measured at the time of transplanting (120 DAS) using a leaf area meter, and the average leaf area per plant was expressed in square centimeters.

3. Results

3.1 Days Taken to Start Germination

The data presented in Table 1 clearly indicated that the number of days required for initiation of germination was significantly influenced by different seed treatments. The results showed a marked reduction in the time taken for germination initiation under treated seeds compared to the control.

Among all treatments, T_9 (GA_3 @ 200 ppm for 20 hours) recorded the minimum number of days (7.89 days) to start germination, indicating its superior effectiveness in enhancing early germination. This was closely followed by T_8 (GA_3 @ 200 ppm for 10 hours), which required 8.18 days, thus ranking as the second best treatment.

In contrast, the control treatment (T_1) recorded the maximum number of days (11.24 days) for germination initiation, indicating delayed germination in the absence of any seed treatment. The treatments involving cow urine and cow dung showed moderate improvement over control but were comparatively less effective than GA_3 treatments.

3.2 Days Taken to Complete Germination

The same Table 1 further shows pre-sowing treatments effect up to significant extent on days taken to complete seed germination. The maximum duration (21.66 days) was observed in T_9, followed by T_8 and T_{10}. Although these treatments took relatively more time to complete germination, they resulted in better and more uniform germination. The control (T_1) recorded the minimum duration (19.39 days); however, this was associated with poor germination percentage and weak seedlings.

Thus, it can be inferred that treatments with slightly longer germination duration resulted in better seedling establishment and overall performance.

3.3 Germination Percentage

The perusal of data in Table 1 further indicate the pre-sowing treatments effect up to significant extent on the germination percentage of karonda seeds under poly house condition. The highest germination percentage (78.00%) was recorded in T_9, which proved to be the best treatment. The second highest germination (74.67%) was observed in T_8, followed by T_{10}.

Table 1. Germination of seeds of karonda as influenced by cow urine, Cow dung and GA_3

Treatment No.	Treatments	Days taken to start germination	Days taken to complete germination	Germination Percentage
T_1	Control	11.24	19.39	53.34
T_2	Cow urine @ 30% 08 hours	9.84	19.84	56.34
T_3	Cow urine @ 30% 12 hours	10.03	20.21	61.34
T_4	Cow urine @ 30% 16 hours	9.94	20.14	62.34
T_5	Cow urine @ 30% 24 hours	9.93	20.24	64.67
T_6	Cow dung 10 hours	9.99	20.34	65.55
T_7	Cow dung 20 hours	10.39	20.84	67.47
T_8	GA_3 @ 200 ppm 10 hours	8.18	21.41	74.67
T_9	GA_3 @ 200 ppm 20 hours	7.89	21.66	78.00
T_{10}	GA_3 @ 100 ppm 24 hours	8.50	20.63	71.34
	S.Em ±	0.76	0.49	0.47
	C.D. at 5%	2.26	1.48	1.41

The lowest germination percentage (53.34%) was recorded in the control (T_1), indicating poor seed viability and performance under untreated conditions. Organic treatments such as cow urine and cow dung improved germination over control but remained inferior to GA_3 treatments.

3.4 Plant height of Karonda Seedling

After pre-sowing treatment with cow urine, cow dung and GA_3 the plant of karonda seedling was measured under poly house condition at 30, 60, 90 and 120 days after sowing. The critical examination of results trend Table 2 reveal that the seedling height was influenced significant at every stage due to seed treatment with different timing of cow urine cow dung, and PGR (GA_3).

Table 2. Plant height of karonda seedling at different growth intervals as influenced by cow urine, cow dung and plant growth regulator (GA_3)

Treatment No.	Treatments	Plant height (cm)			
		30 DAS	60 DAS	90 DAS	120 DAS
T_1	Control	2.76	3.49	6.44	7.54
T_2	Cow urine @ 30% 08 hours	2.84	4.34	7.14	7.48
T_3	Cow urine @ 30% 12 hours	2.97	5.3	7.64	7.9
T_4	Cow urine @ 30% 16 hours	3.04	5.32	7.36	8.62
T_5	Cow urine @ 30% 24 hours	3.07	5.29	7.66	8.24
T_6	Cow dung 10 hours	3.06	5.27	7.62	8.21
T_7	Cow dung 20 hours	3.09	5.33	7.69	8.39
T_8	GA_3 @ 200 ppm 10 hours	3.26	5.75	8.8	9.72
T_9	GA_3 @ 200 ppm 20 hours	3.59	6.59	9.32	10.19
T_{10}	GA_3 @ 100 ppm 24 hours	3.19	5.44	8.45	9.56
S.Em ±		0.15	0.23	0.50	0.48
CD at 0.05		0.47	0.70	1.49	1.43

At 120 DAS, the maximum plant height (10.19 cm) was recorded in T_9, indicating vigorous plant growth. This was followed by T_8 (9.72 cm) as the second best treatment and T_{10} (9.56 cm).

The minimum plant height (7.54 cm) was observed in the control (T_1), which clearly indicated poor vegetative growth in untreated plants. Treatments involving organic inputs showed moderate growth enhancement but were inferior to GA_3 treatments.

3.5 Diameter of Shoot

In the sequence of periodical observations the diameter of shoot under different treatments was also measured at 30, 60, 90 and 120 DAS stages. The data so obtained were subjected to statistical computation. The critical analysis of data in Table 3 evidently indicate that the diameter of seedling shoot was influenced upto significant extent at every stage as a result of seed treated with different timing of cow urine, cow dung and PGR (GA_3).

Table 3. Diameter of shoot of karonda seedling at different growth intervals as influenced by cow urine, cow dung and plant growth regulator (GA_3)

Treatment No.	Treatment	Diameter of shoot (mm)			
		30 DAS	60 DAS	90 DAS	120 DAS
T_1	Control	0.51	1.08	1.76	2.18
T_2	Cow urine @ 30% 08 hours	0.55	1.51	2.11	2.66
T_3	Cow urine @ 30% 12 hours	0.58	1.66	2.33	2.84
T_4	Cow urine @ 30% 16 hours	0.59	1.71	2.36	2.85
T_5	Cow urine @ 30% 24 hours	0.61	1.75	2.39	2.9
T_6	Cow dung 10 hours	0.6	1.72	2.38	2.89
T_7	Cow dung 20 hours	0.63	2.04	2.42	2.94
T_8	GA_3 @ 200 ppm 10 hours	0.64	2.05	2.76	3.11
T_9	GA_3 @ 200 ppm 20 hours	0.65	2.27	2.91	3.21
T_{10}	GA_3 @ 100 ppm 24 hours	0.61	2.01	2.86	3.1
S.Em ±		0.05	0.04	0.08	0.04
CD at 0.05		0.16	0.12	0.26	0.13

At 120 DAS, the maximum shoot diameter (3.21 mm) was recorded in T_9, followed by T_8 (3.11 mm) and T_{10} (3.10 mm), indicating better stem development under GA_3 treatments. The minimum shoot diameter (2.18 mm) was recorded in the control (T_1), reflecting poor plant vigour and reduced growth. Organic treatments showed intermediate performance.

Table 4. Number of leaves/seedling of karonda as influenced by cow urine, cow dung and plant growth regulator (GA_3)

Treatment No.	Treatments	Number of leaves/seedling			
		30 DAS	60 DAS	90 DAS	120 DAS
T_1	Control	3.27	8.39	11.77	14.52
T_2	Cow urine @ 30% 08 hours	3.42	8.88	13.67	15.88
T_3	Cow urine @ 30% 12 hours	3.49	9.13	15.27	17.74
T_4	Cow urine @ 30% 16 hours	3.57	9.27	15.82	17.97
T_5	Cow urine @ 30% 24 hours	3.63	9.4	16.02	18.2
T_6	Cow dung 10 hours	3.59	9.32	15.88	18.07
T_7	Cow dung 20 hours	3.67	9.47	16.07	18.27
T_8	GA_3 @ 200 ppm 10 hours	4.61	11.4	17.62	19.93
T_9	GA_3 @ 200 ppm 20 hours	4.73	11.52	18.22	21.39
T_{10}	GA_3 @ 100 ppm 24 hours	4.12	11.33	17.27	19.77
S.Em ±		0.03	0.03	0.04	0.04
CD at 0.05		0.10	0.11	0.12	0.12

3.6 Number of Leaves / Seedling

In continuation with the sequence of periodical observations the number of leaves/seedling under different treatments was also counted at 30, 60, 90 and 120 DAS stages under poly house condition. The data on this parameter were subjected to statistical analysis. The minute observation of the data in Table 4 reveal that the number of leaves/seedling was influenced up to significant extent at every stage as a result of seed treated with

different concentration of Cow urine, cow dung and PGR (GA_3). At 120 DAS, the maximum number of leaves (21.39) was recorded in T9, followed by T_8 (19.93) and T_{10} (19.77), indicating enhanced vegetative growth. The minimum number of leaves (14.52) was recorded in the control (T_1), which indicates poor leaf production under untreated conditions.

3.7 Fresh and Dry Weight of Leaves

The fresh and dry weight of leaves/ seedling was recorded under treatment. The data in Table 5 indicate that, these parameters were influenced up to significant extent due to different timing cow urine cow dung and GA_3. The highest fresh weight (1.24 g) was recorded in T_9, followed by T_8 (1.21 g) as the second best treatment. The lowest fresh weight (0.70 g) was recorded in the control (T_1), indicating reduced biomass accumulation in untreated plants.

Table 5. Fresh and dry weight of leaves of karonda as influenced by cow urine , cow dung and plant growth regulator (GA_3)

Treatment No.	Treatments	Fresh weight of leaves (gm)	Dry weight of leaves (gm)
T_1	Control	0.70	0.33
T_2	Cow urine @ 30% 08 hours	0.84	0.38
T_3	Cow urine @ 30% 12 hours	0.94	0.40
T_4	Cow urine @ 30% 16 hours	0.97	0.43
T_5	Cow urine @ 30% 24 hours	0.99	0.45
T_6	Cow dung 10 hours	0.98	0.44
T_7	Cow dung 20 hours	1.00	0.46
T_8	GA_3 @ 200 ppm 10 hours	1.21	0.51
T_9	GA_3 @ 200 ppm 20 hours	1.24	0.57
T_{10}	GA_3 @ 100 ppm 24 hours	1.15	0.46
	S.Em ±	**0.09**	**0.03**
	C.D. at 5%	**0.29**	**0.11**

However, the maximum dry weight (0.57 g) was recorded in T_9, followed by T_8 (0.51 g). The minimum dry weight (0.33 g) was recorded in the control (T_1), indicating lower dry matter accumulation.

4. Discussion

The data presented in Table 1 clearly indicated that the number of days required for initiation of germination was significantly influenced by different seed treatments. The results showed a marked reduction in the time taken for germination initiation under treated seeds compared to the control. Among all treatments, T_9 recorded the minimum number of daysto start germination, indicating its superior effectiveness in enhancing early germination. This was closely followed by T_8 which required days, thus ranking as the second best treatment. In contrast, the control treatment (T_1) recorded the maximum number of days for germination initiation, indicating delayed germination in the absence of any seed treatment. The treatments involving cow urine and cow dung showed moderate improvement over control but were comparatively less effective than GA_3 treatments Meng *et al.* (2009). The days taken for initiation (starting) germination is less in seeds soaked in water (T_1) may be due to soaking the seeds in water helped in softening the seed coats, removal of inhibitors and reduced the time required for germination (Bhavya *et al*., 2017).

However the germination percentage was low to complete germination with us of T_6 and T_7. This may be due to either hardness of seed-coat, presence of germination inhibitors or physiological dormancy. With respect to seed germination, under pre-sowing treatment with 200 ppm GA_3 attained the intermediate position. These results are in consonance with those of Kalabandi *et al*. (2003), Bhavya *et al*. (2017) and Pal *et al*. (2019).

The data summarized in Tables 2 to 5 evidently indicated that, the T_9 encouraged the plant height upto maximum extent 30, 60, 90 and 120 days after sowing. The boosted vegetative growth (plant height and number of leaves/seedling) due to pre-sowing seeds treated with 200 ppm GA_3 may be because of the fact that, this plant growth regulator promoted plant growth by ensuring high number of greener leaves with increased

photosynthesis as a result of (i) increase metabolism of the absorbed plant nutrients (ii) influencing cell membranes of leaves, (iii) forming longer and stronger roots to absorb water and nutrients, and (iv) as a result of acting on dividing cells in roots and shoots. Profuse root development ensured more absorption of minerals and soil moisture from the deeper soil layers. These favourable soil conditions brought about efficient utilization of plant nutrients accompanied by activating plant enzymes. These results are also in congruence with the findings of Kalabandi *et al.* (2003), Venkatrao & Reddy (2005), Anburani and Shakila (2010), Al-shahawary *et al.* (2014), Bhavya *et al.* (2017), Patil *et al.* (2018) and Mane *et al.* (2018).

The diameter of shoot of karonda seedling was augmented upto maximum extent when pre-sowing seeds were treated with T_9 at 30, 60, 90 and 120 days after sowing. This was closely followed by 200 ppm GA_3 10 hours. This plant growth regulator improved seedling emergence and growth rate due to enhanced supply of soluble carbohydrates to the growing embryo, which was caused by an increase in x-amylase activity. Rapid and uniform emergence and growth of seedling due to seed priming with GA_3 enabled the plants to use available resources efficiently, leaching to increase in plant biomass including diameter of shoot. These findings corroborate with those of Brijwal and Kumar (2013), Parmar *et al.* (2016) and Bhavya *et al.* (2017).

Due to maximum increase in number of leaves/seedling due to pre- sowing treatment of seeds with 200 ppm GA_3 the leaf area as well as fresh and dry weight of leaves/seedling were automatically increased upto maximum extent. In this way, GA_3 performed the best in increasing these parameters as compared to cow dung and cow urine applied in their different concentrations. These results conform to the findings of Venkatrao & Reddy (2005), Al- shahawary *et al.* (2014) Parmar *et al.* (2016) and Mane *et al.* (2018).

5. Conclusion

The study concludes that pre-sowing seed treatments significantly improved germination and seedling growth of karonda under polyhouse conditions. Among all treatments, T_9 (GA_3 @ 200 ppm for 20 hours) was found to be the most effective, resulting in early and uniform germination, higher germination percentage and enhanced seedling vigour. GA_3 treatments performed better than organic treatments and control due to their role in breaking dormancy and promoting physiological activities. Hence, seed soaking in GA_3 @ 200 ppm for 20 hours is recommended for achieving better germination and healthy seedling development in karonda.

6. Limitation of the Study

Limited studies have compared cow-based organic inputs with plant growth regulators under protected (polyhouse) conditions in karonda. There is also a lack of standardized doses and treatment durations and insufficient information on their comparative effectiveness in improving both germination and seedling growth.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Acknowledgements

We thank the Career Point University for provided all necessary facilities required for fulfillment of this research work.

Competing Interests

Authors have declared that no competing interests exist.

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