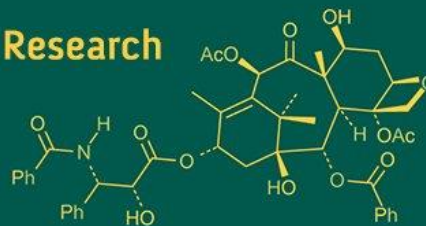


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Growth, seedling vigour and biomass response of five locally available leafy vegetable microgreens to GA₃ and NAA under protected cultivation

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Abstract

Microgreens are increasingly recognised as short-duration, high-value crops suited to protected cultivation, and current commercial interest is often centred on premium or specialised species, while common, locally cultivated leafy vegetables remain comparatively unexplored. This study evaluated the effect of gibberellic acid (GA₃) and naphthalene acetic acid (NAA), applied singly and in combination, on germination and early seedling growth of five locally relevant leafy vegetable microgreens—coriander, fenugreek, spinach, red amaranth and green amaranth—grown under protected cultivation. Four treatments, water spray (T₁), GA₃ at 20 ppm (T₂), NAA at 10 ppm (T₃) and GA₃ at 20 ppm plus NAA at 10 ppm (T₄), were compared in a completely randomized design, with data pooled across two growing seasons. Germination percentage and days to germination varied mainly with crop and were not significantly affected by treatment. In contrast, T₄ consistently produced the greatest plant height, hypocotyl length, root length, fresh weight and dry weight across all five crops, showing that the combined hormonal treatment influenced post-germination growth more than germination itself. Fenugreek recorded the highest overall biomass and root length among the crops tested. Crop-specific differences in response to GA₃ and NAA indicate that locally available leafy vegetables should be evaluated individually rather than assumed to respond uniformly to a common hormonal regime. These findings suggest that common, locally cultivated leafy vegetables can serve as accessible options for microgreen production, offering practical relevance for local growers, although wider evaluation across additional seasons, locations and crop species would be needed before broader recommendations can be made.

Keywords: Microgreens, leafy vegetables, GA₃, NAA, seedling vigour, biomass

1. Introduction

Microgreens are young vegetable seedlings harvested at an early developmental stage, generally within one to three weeks of sowing, and have attracted attention for their nutrient density and bioactive compound content in addition to their short production cycle [1, 2]. Their compact growth habit and rapid turnover make them well suited to intensive, space-efficient production systems, including protected cultivation, and have contributed to their growing recognition as a functional and fresh food category [3, 4].

Alongside this nutritional interest, microgreens are increasingly valued specifically as short-duration crops that allow multiple production cycles within a limited cultivation area and time, a feature that has encouraged their evaluation across a range of substrates and production environments [5, 6].

Much of the existing work on microgreen production has, however, focused on premium, specialised or commercially popular species, including several exotic and branded microgreen types evaluated for their distinct visual, flavour or compositional attributes [7, 8]. Comparatively less attention has been directed towards common, locally cultivated leafy vegetables, even though such crops are widely grown, familiar to local growers and already integrated into regional cropping systems [9, 10].

Locally available leafy vegetables may offer practical advantages for microgreen production, including ready accessibility of seed, adaptation to local agro-climatic conditions and suitability for small-scale or localized protected cultivation systems [10, 11]. Realising this potential, however, requires a clear understanding of how such crops behave specifically at

the microgreen stage, since growth behaviour under short-duration, high-density microgreen production can differ from that observed in conventional field or nursery cultivation, and different crops are likely to show distinct growth responses that need to be compared under common experimental conditions.

Plant growth regulators are commonly used to modify seedling establishment and early growth. Gibberellins (GA_3) are closely associated with cell elongation and shoot development, while auxins such as naphthalene acetic acid (NAA) influence cell division, elongation and root formation [12, 13]; their combined application has been reported to enhance lateral root initiation and elongation through complementary hormonal action [14]. Application of these regulators at suitable concentrations can therefore be expected to modify the growth pattern and biomass accumulation of young seedlings, although the extent of this response is likely to vary among crop species.

Despite the growing interest in microgreens, there remains a research gap regarding the comparative evaluation of common, locally relevant leafy vegetables-as distinct from premium or specialised microgreen species-under a shared set of growth-regulator treatments and cultivation conditions. Coriander, fenugreek, spinach and the amaranths are widely grown leafy vegetables in many regions and are agronomically suited to microgreen production, yet their

differential response to GA_3 and NAA at the microgreen stage has not been systematically compared [9].

The present study was therefore undertaken to evaluate the effect of GA_3 and NAA, applied individually and in combination, on germination, seedling growth, root development and biomass production of five locally relevant leafy vegetable microgreens-coriander, fenugreek, spinach, red amaranth and green amaranth-under protected cultivation. The novelty of the study lies in the comparative evaluation of these five locally available crops under common experimental conditions, generating evidence on their differential response to GA_3 and NAA and their suitability as practical, accessible alternatives to premium microgreen species for localized production systems.

2. Materials and Methods

2.1 Experimental site, period and plant material

The experiment was conducted at the Centre of Excellence on Protected Cultivation and Precision Farming, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India. Five leafy vegetables-coriander (*Coriandrum sativum* L.), fenugreek (*Trigonella foenum-graecum* L.), spinach (*Spinacia oleracea* L.), red amaranth (*Amaranthus cruentus* L.) and green amaranth (*Amaranthus viridis* L.)-were evaluated as microgree



Fig 1: Experimental workflow for microgreen cultivation under protected conditions

2.2 Growing medium, experimental design and treatments

The crops were grown in a cocopeat: vermicompost mixture (4:1, v/v) under protected cultivation. The experiment was laid out in a Completely Randomized Design (CRD) with four treatments and four replications. The treatments were:

T₁ water spray (control); T₂, GA_3 @ 20 ppm; T₃, NAA @ 10 ppm; and T₄, GA_3 @ 20 ppm + NAA @ 10 ppm. Plant growth regulators were applied as a single foliar spray after seedling establishment, at 2 days after germination. Microgreens were harvested for recording of observations at 15 days after sowing.

2.3 Observations recorded

Seven traits were recorded for each treatment and crop: germination percentage, days to germination, plant height, hypocotyl length, root length, fresh weight and dry weight. Germination percentage was calculated as the proportion of seeds that germinated relative to the total number sown, expressed on a percentage basis, and days to germination was recorded as the number of days from sowing to visible seedling emergence.

Plant height, hypocotyl length and root length were measured using a graduated scale on randomly selected seedlings at harvest. Fresh weight was recorded immediately after harvest using an electronic balance, while dry weight was recorded after oven-drying the samples until a constant weight was obtained.

2.4 Statistical analysis

Data were recorded in two independent experimental seasons, and only the pooled data across the two seasons are presented in this manuscript. Treatment means for each crop were compared using the standard error of mean (SEM $\pm$) and the critical difference (CD) at the 5% probability level, following the standard pooled analysis of variance procedure applicable to a Completely Randomized Design. Where the difference between treatment means was smaller than the corresponding CD value, the effect was considered non-significant and is denoted as NS in the tables. No factorial analysis of season $\times$ treatment interaction, post-hoc multiple range test (e.g., Tukey's or Duncan's test), correlation analysis or principal component analysis was performed; only the main effects of treatment, based on the pooled analysis, are reported.

3. Results and Discussion

3.1 Germination percentage

Pooled germination percentage showed considerable variation among crops, while treatment effects were comparatively small (Table 1). Coriander ranged from 77.88 to 79.25%, with the highest numerical value under T₄. Fenugreek recorded the highest overall germination percentage at 91.38% under the T₃ treatment. Meanwhile, spinach and green amaranth showed little variation at around 85.50% and 87.50%, respectively, across treatments, and red amaranth ranged from between 84.38% and 84.88%. Since the pooled treatment differences were not statistically significant for any of the crops (CD, NS), it's clear that germination percentages were mainly influenced by inherent crop characteristics traits rather than the PGR treatments. This matches previous findings that hormonal treatments usually don't do much for final germination rates in leafy greens, were more evident during later stages of seedling growth instead^[12, 13]. Fenugreek's strong germination rate above 90% points to quick, uniform water absorption, whereas coriander lags behind a bit due to its tougher seed coat. Practically speaking, this means seed quality and inherent crop characteristics-rather than PGR application-should be the main focus when aiming for uniform germination in local microgreen production.

3.2 Days to germination

Days to germination was determined mainly by crop type (Table 2). Coriander required the longest period (7.75 days), consistent with its comparatively hard seed coat, whereas fenugreek germinated fastest (2.50 days). Spinach ranged from 4.50 to 4.75 days, red amaranth from 3.62 to 3.88 days

and green amaranth from 3.38 to 3.75 days. Treatment differences were non-significant in all crops, reinforcing that the timing of germination in these species is largely under crop-specific control, with the influence of GA₃ and NAA expressed more clearly during subsequent seedling growth than during germination itself^[12, 13]. This indicates that, for these five crops, differential PGR scheduling is unlikely to be necessary to influence germination speed.

3.3 Plant height

Plant height showed a consistent, statistically significant positive response to the combined treatment in all five crops (Table 3). T₄ recorded the maximum pooled height in coriander (4.28 cm), fenugreek (6.06 cm), spinach (6.39 cm), red amaranth (4.94 cm) and green amaranth (4.99 cm), with the numerical order generally following T₄ > T₃ > T₂ > T₁. This pattern is consistent with the complementary physiological roles of gibberellin in promoting cell elongation and auxin in supporting coordinated shoot growth^[12, 14]. Spinach and fenugreek showed the largest absolute gains in height under T₄ relative to the control, indicating a comparatively stronger post-germination elongation response in these two crops than in coriander and the amaranths, and pointing to fenugreek and spinach as crops particularly responsive to combined PGR application for canopy development at the microgreen stage.

3.4 Hypocotyl length

Hypocotyl length generally increased with PGR application, with T₄ recording the highest pooled value in all five crops (Table 4). The response was non-significant in coriander but significant in fenugreek, spinach, red amaranth and green amaranth, based on the supplied CD values. This crop-specific pattern indicates that GA₃-mediated hypocotyl elongation, well documented in other seedling systems^[13], is not uniformly expressed across leafy vegetable species, reinforcing the need to evaluate each crop individually rather than assuming a common hormonal response across locally available leafy vegetables^[9].

3.5 Root length

Root length increased progressively from T₁ to T₄ in all crops, with fenugreek recording the longest roots overall (11.28 cm under T₄), followed by coriander, spinach, and the two amaranths (Table 5). This pattern is consistent with the established role of auxin, and its interaction with gibberellin, in promoting lateral root initiation and elongation^[14]. Fenugreek showed a strong root response along with higher biomass accumulation, as discussed in Sections 3.6 and 3.7. These results indicate that fenugreek showed a particularly strong response to the combined GA₃ and NAA treatment among the crops tested. to the combined GA₃ and NAA treatment, making it a suitable crop for further evaluation in local microgreen production. for local microgreen production^[9].

3.6 Fresh weight and 3.7 Dry weight

PGR application also influenced overall biomass production. The treated microgreens generally had higher fresh and dry weights, with both traits hitting their highest numbers when GA₃ and NAA were used together (T₄) across all five crops. This pattern was similar to that observed for plant height and root growth, suggesting an overall improvement in seedling development under the combined treatment. This concurrent

improvement across shoot elongation, root development and biomass accumulation is consistent with reports linking combined hormonal treatments to overall gains in seedling vigour and biomass in microgreen and vegetable seedling systems [6, 15]. Fenugreek recorded the highest fresh weight (1.34 g) and dry weight (0.22 g) among the tested crops, confirming it as the strongest overall performer under the combined treatment, while red and green amaranth, despite showing statistically significant relative increases in most traits, recorded the lowest absolute biomass, reflecting their comparatively smaller seedling size at the microgreen stage. Fresh weight in red amaranth was the only case where the treatment effect was non-significant (CD, NS), indicating a comparatively weaker biomass response to PGR application in this crop specifically. Overall, these results show that measurable improvements in seedling vigour and biomass accumulation, comparable in direction to those reported for other microgreen production systems [5, 6], can be achieved using commonly available, locally cultivated leafy vegetable crops rather than specialised or premium microgreen species [3, 4]. This supports the practical relevance of evaluating locally accessible crop resources for microgreen production, although the present study did not include a direct comparison with premium or exotic microgreen species and therefore does not establish superiority over them.

3.8 Comparative crop performance and practical implications

Across all measured traits, the magnitude of response to GA₃ and NAA differed among the five crops, with fenugreek and spinach showing the strongest post-germination growth and biomass gains, while red and green amaranth showed comparatively smaller absolute increases despite statistically significant treatment effects for most traits. Germination-related traits (germination percentage and days to germination) remained largely unaffected by treatment in all crops, whereas post-germination traits-plant height, hypocotyl length, root length and biomass-were consistently more responsive, particularly under the combined GA₃ + NAA treatment. This crop-dependent pattern indicates that locally available leafy vegetables should not be assumed to respond uniformly to a standard PGR regime, and that crop-specific evaluation is necessary when developing PGR-based protocols for local microgreen production [9, 12]. Taken together, the results demonstrate that meaningful gains in seedling growth and biomass can be obtained using common, locally cultivated leafy vegetable crops, supporting their consideration as accessible options for local microgreen production; however, the economic and large-scale practical feasibility of such systems was not assessed in this study and would require further evaluation.

Table 1: Effect of GA₃ and NAA on germination percentage of five leafy vegetable microgreens (pooled data over two seasons)

Treatment	Coriander	Fenugreek	Spinach	Red amaranth	Green amaranth
T ₁ -Water spray (control)	77.88	90.75	85.50	84.38	87.50
T ₂ -GA ₃ @ 20 ppm	78.62	91.00	85.50	84.38	87.50
T ₃ -NAA @ 10 ppm	79.12	91.38	85.50	84.88	87.50
T ₄ -GA ₃ @ 20 ppm + NAA @ 10 ppm	79.25	91.12	85.50	84.62	87.50
SEm±	0.48	0.36	0.65	0.56	0.65
CD (5%)	NS	NS	NS	NS	NS

Unit: %. Values are pooled means over two experimental seasons. NS = non-significant at 5% probability level.

Table 2: Effect of GA₃ and NAA on days to germination of five leafy vegetable microgreens (pooled data over two seasons)

Treatment	Coriander	Fenugreek	Spinach	Red amaranth	Green amaranth
T ₁ -Water spray (control)	7.75	2.50	4.50	3.62	3.62
T ₂ -GA ₃ @ 20 ppm	7.75	2.50	4.75	3.62	3.75
T ₃ -NAA @ 10 ppm	7.75	2.50	4.75	3.88	3.62
T ₄ -GA ₃ @ 20 ppm + NAA @ 10 ppm	7.75	2.50	4.75	3.88	3.38
SEm±	0.25	0.29	0.24	0.28	0.19
CD (5%)	NS	NS	NS	NS	NS

Unit: days. Values are pooled means over two experimental seasons. NS = non-significant at 5% probability level.

Table 3: Effect of GA₃ and NAA on plant height (cm) of five leafy vegetable microgreens (pooled data over two seasons)

Treatment	Coriander	Fenugreek	Spinach	Red amaranth	Green amaranth
T ₁ -Water spray (control)	3.29	5.16	5.25	4.38	4.36
T ₂ -GA ₃ @ 20 ppm	3.60	5.51	5.69	4.64	4.65
T ₃ -NAA @ 10 ppm	3.97	5.75	6.11	4.82	4.88
T ₄ -GA ₃ @ 20 ppm + NAA @ 10 ppm	4.28	6.06	6.39	4.94	4.99
SEm±	0.09	0.09	0.09	0.07	0.08
CD (5%)	0.28	0.27	0.29	0.21	0.25

Unit: cm. Values are pooled means over two experimental seasons. NS = non-significant at 5% probability level.

Table 4: Effect of GA₃ and NAA on hypocotyl length (cm) of five leafy vegetable microgreens (pooled data over two seasons)

Treatment	Coriander	Fenugreek	Spinach	Red amaranth	Green amaranth
T ₁ -Water spray (control)	3.18	4.37	3.17	4.13	4.19
T ₂ -GA ₃ @ 20 ppm	3.23	4.47	3.32	4.12	4.29
T ₃ -NAA @ 10 ppm	3.23	4.60	3.53	4.25	4.39
T ₄ -GA ₃ @ 20 ppm + NAA @ 10 ppm	3.33	4.74	3.67	4.36	4.52
SEm±	0.09	0.02	0.02	0.03	0.02
CD (5%)	NS	0.07	0.07	0.08	0.07

Unit: cm. Values are pooled means over two experimental seasons. NS = non-significant at 5% probability level.

Table 5: Effect of GA₃ and NAA on root length (cm) of five leafy vegetable microgreens (pooled data over two seasons)

Treatment	Coriander	Fenugreek	Spinach	Red amaranth	Green amaranth
T ₁ -Water spray (control)	8.38	9.73	7.83	6.41	6.42
T ₂ -GA ₃ @ 20 ppm	8.65	10.30	8.31	6.65	6.66
T ₃ -NAA @ 10 ppm	9.09	10.66	8.75	6.96	7.01
T ₄ -GA ₃ @ 20 ppm + NAA @ 10 ppm	9.35	11.28	9.39	7.28	7.31
SEm±	0.09	0.13	0.13	0.16	0.13
CD (5%)	0.29	0.39	0.39	0.48	0.39

Unit: cm. Values are pooled means over two experimental seasons. NS = non-significant at 5% probability level.

Table 6: Effect of GA₃ and NAA on fresh weight (g) of five leafy vegetable microgreens (pooled data over two seasons)

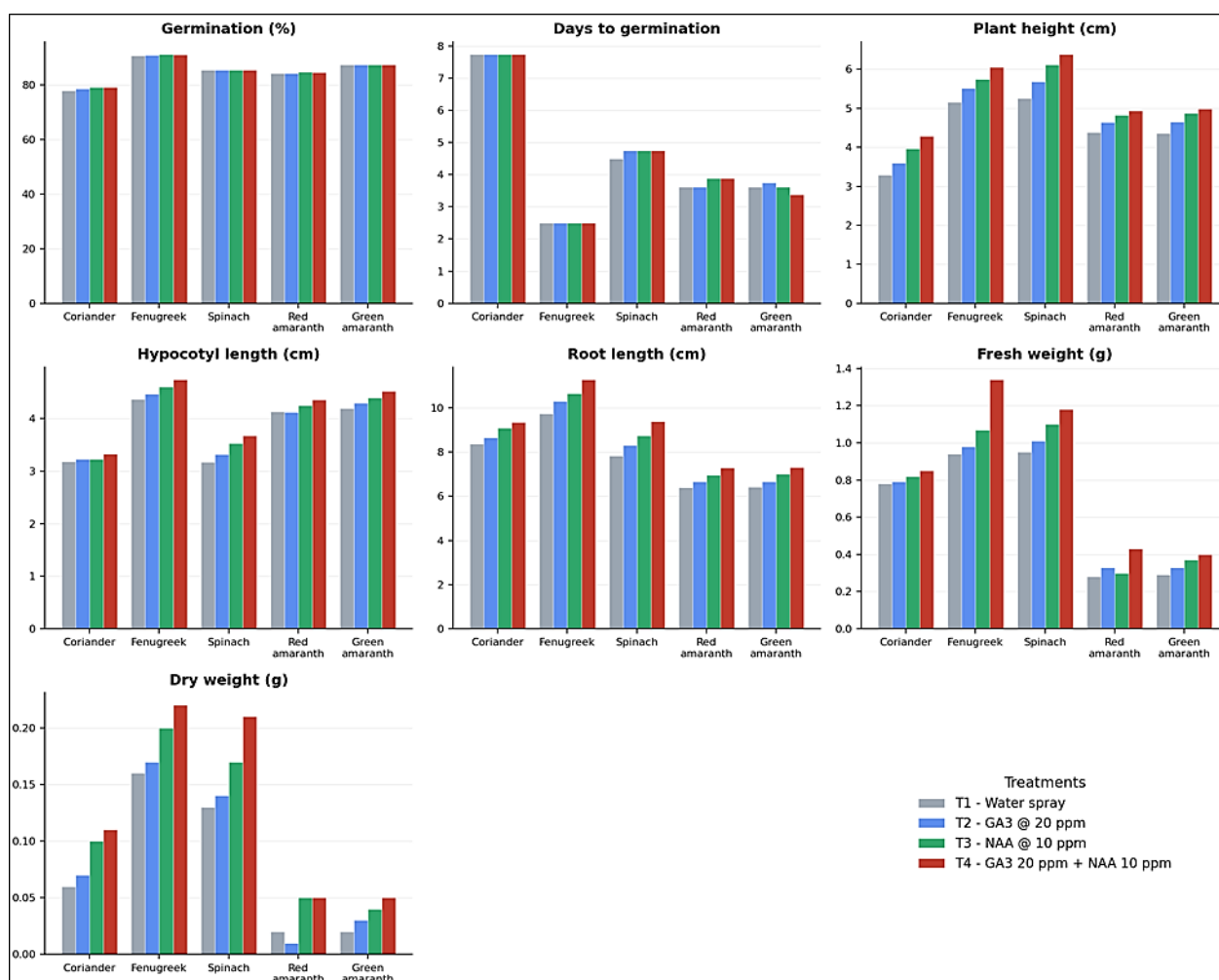
Treatment	Coriander	Fenugreek	Spinach	Red amaranth	Green amaranth
T ₁ -Water spray (control)	0.78	0.94	0.95	0.28	0.29
T ₂ -GA ₃ @ 20 ppm	0.79	0.98	1.01	0.33	0.33
T ₃ -NAA @ 10 ppm	0.82	1.07	1.10	0.30	0.37
T ₄ -GA ₃ @ 20 ppm + NAA @ 10 ppm	0.85	1.34	1.18	0.43	0.40
SEm±	0.01	0.03	0.02	0.04	0.01
CD (5%)	0.02	0.10	0.05	NS	0.03

Unit: g. Values are pooled means over two experimental seasons. NS = non-significant at 5% probability level.

Table 7: Effect of GA₃ and NAA on dry weight (g) of five leafy vegetable microgreens (pooled data over two seasons)

Treatment	Coriander	Fenugreek	Spinach	Red amaranth	Green amaranth
T ₁ -Water spray (control)	0.06	0.16	0.13	0.02	0.02
T ₂ -GA ₃ @ 20 ppm	0.07	0.17	0.14	0.01	0.03
T ₃ -NAA @ 10 ppm	0.10	0.20	0.17	0.05	0.04
T ₄ -GA ₃ @ 20 ppm + NAA @ 10 ppm	0.11	0.22	0.21	0.05	0.05
SEm±	0.01	0.01	0.01	0.00	0.01
CD (5%)	0.02	0.03	0.03	0.01	0.02

Unit: g. Values are pooled means over two experimental seasons. NS = non-significant at 5% probability level.

**Fig 2:** Effect of GA₃ and NAA on germination, growth and biomass parameters of five leafy vegetable microgreens (pooled data)

4. Conclusion

Based on pooled observations from two experimental seasons, the combined application of GA₃ @ 20 ppm + NAA @ 10 ppm (T₄) produced the most consistent improvement in plant height, hypocotyl length, root length, fresh weight and dry weight across all five leafy vegetable microgreens, while germination percentage and days to germination remained comparatively stable across treatments. The magnitude of response varied among crops, with fenugreek showing particularly strong biomass and root development, and spinach also showing a marked height and biomass response, whereas red and green amaranth showed comparatively smaller absolute gains. These findings indicate that combined GA₃ and NAA application can enhance post-germination seedling vigour and biomass in leafy vegetable microgreens, and that common, locally available and commonly cultivated leafy vegetable crops can be considered for microgreen production under protected cultivation. Crop selection for local microgreen production should be based on local availability, observed growth performance and specific production objectives, and the results support further utilization and evaluation of locally available crop resources for localized, potentially low-cost microgreen production systems, rather than establishing their universal superiority over premium or exotic microgreen species.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Urvashi Verma conceptualized and conducted the experiment, collected and analysed the data, and prepared the original manuscript. Dr. Annu Verma contributed to the experimental design, interpretation of the results, critical revision of the manuscript and overall supervision of the research. All authors read and approved the final manuscript.

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