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IMPACT OF BOTANICAL-BASED NANO-FORMULATIONS AS SEED PRIMING ON GERMINATION AND SEEDLING QUALITY IN OKRA (*ABELMOSCHUS ESCULENTUS* L.)

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ABSTRACT

An experiment was conducted at Department of Nanotechnology, Anand Agricultural University, Anand with a view to study the impact of botanical-based nano-formulations as seed priming on germination and seedling quality in okra (*Abelmoschus esculentus* L.) during the summer, 2023. The experiment was arranged in completely randomized design (CRD) with three repetition having twenty five seed priming treatments (**T_1:** Control, **T_2:** 5% GNF, **T_3:** 10% GNF, **T_4:** 15% GNF, **T_5:** 20% GNF, **T_6:** 5% MNF, **T_7:** 10% MNF, **T_8:** 15% MNF, **T_9:** 20% MNF, **T_{10}:** 5% GNF + 5% MNF, **T_{11}:** 5% GNF + 10% MNF, **T_{12}:** 5% GNF + 15% MNF, **T_{13}:** 5% GNF + 20% MNF, **T_{14}:** 10% GNF + 5% MNF, **T_{15}:** 10% GNF + 10% MNF, **T_{16}:** 10% GNF + 15% MNF, **T_{17}:** 10% GNF + 20% MNF, **T_{18}:** 15% GNF + 5% MNF, **T_{19}:** 15% GNF + 10% MNF, **T_{20}:** 15% GNF + 15% MNF, **T_{21}:** 15% GNF + 20% MNF, **T_{22}:** 20% GNF + 5% MNF, **T_{23}:** 20% GNF + 10% MNF, **T_{24}:** 20% GNF + 15% MNF, **T_{25}:** 20% GNF + 20% MNF). The results revealed that the seed treatments with 20% GNF + 15% MNF (T_{24}) differentially proved to be significantly superior in seed germination (%), seedling shoot length, seedling root length and seedling vigour index-II. Other T_3 differentially proved to be significantly superior in seedling length, seedling fresh weight and seedling vigour index-I of okra. Where, GNF: Gliricidia Nano formulation and MNF: Moringa Nano formulation.

***Keywords*:** Seed priming, Botanical based extract, GNF, MNF.

Introduction

Okra (*Abelmoschus esculentus*), commonly known as lady's finger, bhindi, or gumbo, is a seed-propagated crop well-suited to warm climates. It is highly sensitive to frost, low temperatures (below 15°C), waterlogging, and drought. Okra is predominantly cultivated in tropical and subtropical regions, and also in the warmer zones of temperate regions. The crop flourishes in hot and humid conditions, with optimal growth and fruiting observed at average temperatures around 25°C and relative humidity ranging from 65–85%. Seed germination in okra is most efficient at temperatures between 30–35°C. When temperatures fall below 25°C, germination slows down considerably, while temperatures above 42°C negatively impact plant and fruit development, causing desiccation, drying and premature dropping of flower buds.

Now a days, farmers and researchers put high attention to biostimulant to improve agricultural sustainability, however, other natural products should be considered, studied, and assessed and the present review intends to highlight the importance of plant extract to improve agricultural sustainability and in particular crops quality and quantity. Primary and secondary plant metabolites affect important biological activities influencing plant physiological responses and plant phenotype. Several previous studies reported the effects of plant extracts on hormones and polyphenols (Lucini *et al.,* 2018), organic acids and sugars contents (Abou Chehade *et al.,* 2018).

Studies on effect of different plant extracts on crop productivity have been carried out by various workers, but the field is still in developing stages and still more research needs to be done in the area to explore more and more plants and plant products with the aim to develop a low-cost, effective and potent growth promoter with little or no side effects form medicinal and aromatic plants. The development of botanical based growth promoter is necessary to stimulate physiological processes in plant and ultimately increase the productivity and quality of crop and also environmentally friendly. The objectives of this investigation are to identify the plant species which can act as growth promoter and development of effective formulations from combination of medicinal plants to increase the quality and productivity in okra. Moringa leaf extract is used as an effective plant growth hormone to enhance seed germination, improving yield and growth in plants (Phiri, 2010).

Nanotechnology is one of the latest platforms to achieve these goals. Nanotechnologies offer novel and unanticipated possibilities because of the new properties and behaviors of nanomaterials, which can be exploited using a structured and planned approach. Nanotechnology is contributing immensely to the various branches of science, engineering and technology namely, physical sciences, chemical sciences, agriculture, food science, medicine, environmental science, information technology and energy science (Hui and Sherkat, 2005; Kumar *et al.,* 2019; Wang *et al.,* 2011).

Materials and Methods

The experiment consisted of twenty five seed priming methods (Control, 5% GNF, 10% GNF,15% GNF, 20% GNF,5% MNF, 10% MNF, 15% MNF, 20% MNF, 5% GNF + 5% MNF, 5% GNF + 10% MNF, 5% GNF + 15% MNF, 5% GNF + 20% MNF, 10% GNF + 5% MNF, 10% GNF + 10% MNF, 10% GNF + 15% MNF, 10% GNF + 20% MNF, 15% GNF + 5% MNF, 15% GNF + 10% MNF, 15% GNF + 15% MNF, 15% GNF + 20% MNF, 20% GNF + 5% MNF, 20% GNF + 10% MNF, 20% GNF + 15% MNF, 20% GNF + 20% MNF) and Okra seed were collected from Main Vegetable Research Station, Anand Agricultural University, Anand. Therefore, there were 25 treatments in the factorial arrangement. Except for the control treatment, Okra seed were treated with each seed priming method for 24 hours and shade dried for 6 hours before sowing. The experiment was conducted in summer 2023 in a laboratory at Department of nanotechnology, anand Agricultural University, Anand, in a completely randomized design (CRD) with 3 replicates. For each replicate, hundred seeds were used per treatment. A total of three hundred seeds (three replications of 100 seeds) were used in the germination test for each treatment. In sterile petri dishes, hundred seeds from each treatment were placed on filter paper that had been moistened with distilled water. Finally, the petri dishes were placed in a growth chamber at 25°C for 21 days, and the seeds were then kept moist with distilled water for 30 days in the laboratory. Germinated seedlings were counted starting with the first seedling that germinated and ending 21 days after placing the seeds in the petri dish.

Result and Discussion

Germination (%)

The data regarding to germination (%) of okra as influenced by seed priming with nano formulation are depicted in table 1. The observations on seed germination indicated that the germination percentage of okra seed was significantly influenced by different seed priming treatments. Among the various treatments, seed priming with 20% GNF + 15% MNF (T_{24}) recorded significantly the highest seed germination (90.00 %) during the year 2023 respectively. However, it remained at par with the treatments T_3 (89.88%), T_{20} (89.44%), T_{23} (88.89%), T_{19} (87.21%) and T_{15} (86.54%) during the year 2023 respectively. Significantly the lowest seed germination was recorded in the control treatment T_1 (60.00%) during the year 2023 respectively.

Enhanced germination observed under GNF and MNF treatments may be attributed to the presence of bioactive compounds such as flavonoids, phenolics and growth-promoting phytohormones, which play a vital role in stimulating seed metabolism and vigor. Makkar and Becker (1996) similarly reported that seed treatments with *Moringa oleifera* extracts significantly improved germination and early seedling growth, attributing the effect to bioactives like zeatin and natural antioxidants. These compounds are known to modulate physiological processes such as cell division and enzymatic activation during early germination. Furthermore, biopriming with *Gliricidia sepium* based formulations has also been found to enhance germination by improving seed metabolic readiness and promoting beneficial microbial activity in the rhizosphere. This finding is supported by the work of Moktar *et al.* (2012), who demonstrated improved germination and seedling establishment in cowpea treated with gliricidia derived biostimulants.

The combined effects of biochemical stimulation and rhizospheric enhancement thus appear to be a key mechanism behind the superior germination and seedling traits observed in GNF and MNF treatments.

Seedling shoot length (cm)

The data presented in Table 1 revealed that there were significant differences in seedling shoot length (cm) of okra due to various treatments. Among the various treatments, seed priming with 20% GNF (T_5) recorded significantly higher shoot length (17.99 cm). It was remained at par with the seed priming treatment T_5 (17.99 cm), T_{24} (17.95 cm), T_{15} (17.88 cm), T_{23} (17.85 cm) and T_{19} (17.59 cm) during the year 2023, respectively. Significantly the lowest shoot length (6.84 cm) during the year 2023 respectively was noticed in control (T_1).

The increased shoot growth observed in treatments involving nano-formulations may be attributed to enhanced cell elongation and hormonal activity triggered by the bioactive components of the applied formulations. Nano-encapsulated nutrients can improve nutrient uptake efficiency and stimulate endogenous hormone synthesis, particularly auxins and cytokinins, which are known to promote shoot elongation. Comparable findings were reported by Foidl *et al.* (2001), who demonstrated that foliar application of *moringa oleifera* leaf extract significantly enhanced shoot elongation in various crops, primarily due to its rich content of zeatin a natural cytokinin and other growth-enhancing substances. These results suggest that the nano-formulations used in the present study may mimic or amplify similar physiological responses, resulting in vigorous shoot development.

Seedling root length (cm)

The data presented in Table 1 revealed that there were significant differences in seedling root length (cm) of okra due to various treatments. Among the various treatments, seed priming with 20% GNF + 15% MNF (T_{24}) recorded significantly higher seedling root length (11.79 cm) as compared to other treatment. It was remained at par with the seed priming treatment T_4 (11.50 cm), T_{19} (11.25 cm), T_{20} (10.88 cm) and T_{16} (10.81 cm). Significantly the lowest root length (6.96 cm) during the year 2023 respectively was noticed in control (T_1).

Improved root elongation observed in GNF and MNF treatments may be attributed to enhanced nutrient uptake efficiency facilitated by the nano-formulations. These nano-sized particles provide a greater surface area and improved solubility, which facilitate faster and more efficient absorption of nutrients at the root interface. According to Saini *et al.* (2020) nano-formulations significantly influence root morphology by increasing root length, branching, and surface area, thereby improving the plant's ability to access water and essential nutrients from the rhizosphere. The enhanced root architecture not only supports better anchorage but also contributes to overall seedling vigor and establishment.

Seedling length (cm)

The data presented in Table 1 revealed that there were significant differences in seedling length (cm) of okra due to various treatments. Among the various treatments, seed priming with 15% GNF + 10% MNF (T_{19}) recorded significantly higher seedling length (28.84 cm) as compared to other treatment. It was remained at par with the seed priming treatment T_3 (28.83 cm), T_4 (28.68 cm), T_5 (28.63 cm), T_{20} (28.29 cm) and T_{24} (27.63 cm) during the year 2023, respectively. Significantly the lowest seedling length was recorded in the control treatment T_1 (19.97 cm) during the year 2023 respectively.

The improved seedling performance observed under certain treatments may be attributed to the bioactivity of moringa leaf extract (MLE), which is rich in growth-promoting substances such as cytokinins, ascorbates and minerals. According to Rehman *et al.* (2014), MLE application can stimulate earlier emergence and promote vigorous initial seedling growth, leading to enhanced establishment. Similarly, Muhammad *et al.* (2015) reported that MLE positively influenced seed germination and seedling vigor due to its bioactive constituents. In the present study, treatments involving MLE exhibited improved shoot and root lengths, suggesting an active metabolic stimulation during early seedling development. Moreover, the combination of MLE with nano-formulated nutrients (GNF and MNF) may have produced a synergistic effect, as reflected in superior germination percentages and total seedling length. This observation aligns with the findings of Soni *et al.* (2024), who demonstrated increased biomass accumulation when nano-biofertilizers were used in combination. Likewise, Basra *et al.* (2011) emphasized the effectiveness of MLE as a natural biostimulant, enhancing seedling growth in spring maize over untreated controls irrigated with tap water. These results collectively support the hypothesis that MLE, particularly when combined with advanced nutrient formulations, significantly improves seedling vigor through both hormonal and nutritional pathways.

Table 1: Effect of nano formulation in seed priming of okra in germination (%), seedling shoot length, seedling root length and seedling length

Treatment	Germination	Seedling shoot length	Seedling root length	Seedling length
	(%)	(cm)		
T_1 - Control	60.00	13.01	6.96	19.97
T_2 - 5% GNF	61.10	13.56	9.51	23.07
T_3 - 10% GNF	89.88	17.04	9.68	28.83
T_4 - 15% GNF	80.00	17.18	11.50	28.68
T_5 - 20% GNF	82.70	17.99	10.54	28.53
T_6 - 5% MNF	62.22	13.97	9.20	23.17
T_7 - 10% MNF	83.16	14.96	10.37	25.33
T_8 - 15% MNF	84.34	16.98	9.98	26.96
T_9 - 20% MNF	61.22	14.74	7.52	22.26
T_{10} - 5% GNF + 5% MNF	71.44	15.67	7.54	23.21
T_{11} - 5% GNF + 10% MNF	72.97	15.72	8.01	23.73
T_{12} - 5% GNF + 15% MNF	69.33	15.42	8.33	23.75
T_{13} - 5% GNF + 20% MNF	63.28	14.43	7.85	22.28
T_{14} - 10% GNF + 5% MNF	68.43	13.98	7.57	21.54
T_{15} - 10% GNF + 10% MNF	86.54	17.88	9.58	27.46
T_{16} - 10% GNF + 15% MNF	85.30	17.35	10.81	28.16
T_{17} - 10% GNF + 20% MNF	71.19	14.62	6.26	20.88
T_{18} - 15% GNF + 5% MNF	60.39	15.00	7.31	22.31
T_{19} - 15% GNF + 10% MNF	87.21	17.59	11.25	28.84
T_{20} - 15% GNF + 15% MNF	89.44	17.41	10.88	28.29
T_{21} - 15% GNF + 20% MNF	65.11	16.54	8.94	25.48
T_{22} - 20% GNF + 5% MNF	69.20	16.12	7.56	23.68
T_{23} -20% GNF + 10% MNF	88.89	17.85	9.75	27.60
T_{24} - 20% GNF + 15% MNF	90.00	17.95	11.79	27.63
T_{25} - 20% GNF + 20% MNF	63.51	15.26	8.57	23.83
S.Em. ±	1.23	0.62	0.34	0.96
C.D. at 5%	3.49	1.75	0.98	2.73
C.V. %	2.85	6.71	6.55	6.66

Seedling fresh weight (gm)

The data presented in Table 2 revealed that there were significant differences in seedling fresh weight (g) of okra due to various treatments. Among the various treatments, seed priming with (T_7) recorded significantly higher seedling length (4.816 g) as compared to other treatment. It was remained at par with the all seed priming treatment except T_2 (4.220 g). Significantly the lowest seedling fresh weight (4.154 g) during the years 2023 respectively was noticed in control (T_1).

The enhanced fresh weight recorded in GNF and MNF treatments can be attributed to improved nutrient availability and uptake efficiency, a result of the nano sized particles in the applied formulations. These nano fertilizers possess high surface-area-to-volume ratios, which facilitate better root absorption, prolonged nutrient release and minimized losses due to leaching or volatilization. This in turn, promotes sustained cellular activity, water retention, and metabolic processes crucial for biomass accumulation. These findings are consistent with the results of Raliya *et al.* (2017), who demonstrated that nano-fertilizers significantly improve plant fresh biomass by enhancing cell expansion and physiological efficiency. The increased fresh weight observed in the present study likely reflects the synergistic effect of improved nutrient assimilation and active physiological functioning triggered by nano interventions.

Seedling dry weight (gm)

The data presented in Table 2 revealed that there were significant differences in seedling dry weight (g) of okra due to various treatments. Among the various treatments, seed priming with 10% GNF + 5% MNF (T_{14}) recorded significantly higher seedling dry weight (0.1802 g) as compared to other treatment. It was remained at par with the all seed priming treatment except T_2 (0.1658). Significantly the lowest seedling dry weight was recorded in the control treatment T_1 (0.1602 g) during the year 2023 respectively.

Nano-formulations are known to facilitate a steady and controlled release of nutrients, which supports prolonged physiological activity and sustained plant growth. This continuous nutrient availability enhances metabolic processes, including photosynthesis and protein synthesis, ultimately leading to greater dry matter accumulation. In the present study, treatments with GNF and MNF showed significant improvement in dry weight, likely due to improved nutrient assimilation and uninterrupted physiological functions. Similar outcomes were reported by Alam *et al.* (2024), who observed increased dry matter content in nano treated seedlings, attributing it to enhanced photosynthetic efficiency, better nutrient uptake, and improved carbon assimilation. These findings support the role of nano fertilizers in promoting robust biomass production through sustained physiological enhancement.

Seedling Vigour Index-I

The seedling vigour Index-I, which integrates both germination and seedling length, ranged from 1198.15 T_1 (control) to a maximum of 2591.54 in T_3 (10% GNF). High values were also seen in treatment T_{20} (2530.31) and T_{19} (2515.37). It was remained at par with the seed priming treatment T_{24} (2486.61), T_{23} (2453.33) and T_{16} (2402.09) during the years 2023, respectively.

The observed improvement in seedling parameters suggests enhanced seed metabolic activity and early establishment vigor as a result of nano-fertilizer treatments. The fine particle size and increased surface reactivity of nano-nutrients likely facilitated better mobilization of seed reserves, rapid enzymatic activation and efficient energy utilization during the early germination phases. This in turn contributed to more vigorous seedling growth and higher seedling vigour index-I. Similar results were reported by Rao *et al.* (2021), who demonstrated that seeds treated with nano-nutrient formulations exhibited significantly higher vigor indices, attributing the enhancement to improved reserve mobilization and accelerated physiological processes during early seedling development.

Seedling Vigour Index-II

Seedling vigour index-II values, which reflect physiological seedling strength based on dry weight, also increased significantly. The maximum value (15.70) was recorded in T_{24} followed closely by T_3 (15.31), T_{23} (15.25) and (T_{20}) (15.52) compared to the T_1 (9.61).

These results are consistent with the findings of Kumar *et al.* (2022), who reported significant improvements in seedling vigour indices following the application of bioactive nano formulations. The elevated seedling vigour index-II scores in the current study underscore the role of nano-formulations in enhancing seedling robustness, nutrient use efficiency, and early establishment potential. Higher vigour indices, particularly under combined treatments, reflect strong metabolic activity and efficient resource mobilization. Nano formulations appear to stimulate both germination and post-germination growth, providing a sustainable and effective bio-enhancement strategy. Similar increases in seedling vigor through the application of *moringa oleifera* and *gliricidia sepium* extracts have been documented by Yasmeen *et al.* (2013), further validating the bio efficacy of such natural inputs.

Across all parameters the application of GNF and MNF especially in combination at 10-15% concentrations, significantly enhanced seedling quality over the untreated control. The synergistic effect observed aligns with prior studies emphasizing the superior bioavailability and efficiency of nano-formulated nutrients, suggesting their potential in advancing sustainable and precision agriculture practices.

Table 2: Effect of nano formulation in seed priming of okra in Seedling fresh & dry weight (gm) and Seedling vigour index-I & index-II

Treatment	Seedling fresh weight (gm)	Seedling dry weight (gm)	Seedling vigour index-I	Seedling vigour index-II
T_1 - Control	4.154	0.1602	1198.15	9.61
T_2 - 5% GNF	4.220	0.1658	1409.57	10.13
T_3 - 10% GNF	4.790	0.1704	2591.54	15.31
T_4 - 15% GNF	4.300	0.1771	2294.29	14.17
T_5 - 20% GNF	4.429	0.1783	2359.09	14.74
T_6 - 5% MNF	4.355	0.1749	1441.51	10.88
T_7 - 10% MNF	4.816	0.1697	2106.34	14.11
T_8 - 15% MNF	4.388	0.1709	2273.73	14.41

T_9 - 20% MNF	4.451	0.1725	1363.10	10.56
T_{10} - 5% GNF + 5% MNF	4.591	0.1751	1658.14	12.51
T_{11} - 5% GNF + 10% MNF	4.335	0.1708	1731.55	12.46
T_{12} - 5% GNF + 15% MNF	4.477	0.1731	1646.90	12.00
T_{13} - 5% GNF + 20% MNF	4.585	0.1721	1410.09	10.89
T_{14} - 10% GNF + 5% MNF	4.348	0.1802	1474.33	12.33
T_{15} - 10% GNF + 10%MNF	4.407	0.1705	2376.28	14.75
T_{16} - 10% GNF + 15%MNF	4.658	0.1688	2402.09	14.40
T_{17} - 10% GNF + 20%MNF	4.519	0.1753	1486.06	12.48
T_{18} - 15% GNF + 5% MNF	4.392	0.1768	1347.19	10.68
T_{19} - 15% GNF + 10%MNF	4.504	0.1693	2515.37	14.77
T_{20} - 15% GNF + 15%MNF	4.602	0.1735	2530.31	15.52
T_{21} - 15% GNF + 20%MNF	4.499	0.1737	1659.03	11.31
T_{22} - 20% GNF + 5% MNF	4.494	0.1723	1638.58	11.92
T_{23} -20% GNF + 10% MNF	4.449	0.1716	2453.33	15.25
T_{24} - 20% GNF + 15%MNF	4.420	0.1745	2486.61	15.70
T_{25} - 20% GNF + 20%MNF	4.712	0.1715	1513.48	10.89
S.Em. ±	0.200	0.006	68.35	0.40
C.D. at 5%	0.567	0.017	194.16	1.12
C.V. %	7.725	5.888	6.25	5.33

Conclusions

The results obtained in the present investigation are discussed earlier and summarized above; the salient findings from the same are concluded as under.

Seed Priming with Nano formulations Enhances Early Seedling Traits

The in vitro study clearly established that seed priming with combined nano formulations (particularly T_{24}: 20% GNF + 15% MNF) significantly improved germination percentage (up to 90%), shoot and root length, seedling fresh and dry weight, and seedling vigour indices. This highlights the effectiveness of nano-priming in improving early growth parameters, likely due to enhanced nutrient and bioactive compound delivery at the seedling stage.

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