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RESEARCH ARTICLE

Nanoformulations of auxin and zeatin: A sustainable approach to minimize flower shedding in greengram

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Abstract

Greengram is a protein-rich pulse crop, facing significant yield challenges due to flower shedding, caused by factors such as limited photosynthesis, hormonal imbalances, genetic traits, etc. The study aimed to develop and evaluate nanoemulsions of 1-Naphthalene acetic acid (NAA) and zeatin to reduce flower shedding and improve crop productivity. Laboratory synthesis and characterization were conducted at the Centre for Agricultural Nanotechnology and a field trial was carried out at the Agricultural Research Station, Bhavanisagar. Nanoemulsions were standardized using 50 % ethanol as the aqueous phase for NAA and 0.25 % DMSO for zeatin, with liquid paraffin and Tween 80 forming the oil phase. The resultant formulations achieved reduced particle sizes (80-300 nm) with higher stability. Field experiment consisted of foliar application of nanoemulsions at varying concentrations and conventional formulations at recommended doses, applied at 50 % flowering and 10 days thereafter. The results demonstrated that NAA nanoemulsion at 30 ppm significantly enhanced leaf area, dry matter production and chlorophyll index. Flower shedding was reduced to 60.2 %, compared to control, while pod setting improved by 47.5 %. The higher seed yield (1096 kg ha^{-1}) and haulm yield were recorded with NAA nanoemulsion at 30 ppm, showing a 30.8 % increase over the control. Conventional zeatin at 5 ppm also performed well but was less effective than NAA nanoemulsion. The study concluded that NAA nanoemulsion at 30 ppm effectively reduces flower shedding and enhances greengram productivity, offering a viable solution for improving sustainable crop management.

Keywords: flower shedding; greengram; NAA; nanoemulsion; zeatin

Introduction

Pulses are a vital component of the human diet and are valued for their high nutritional and protein content. Pulses are rich in fibre, vitamins and minerals and meet about one-third of global dietary protein needs. India is the leading producer and consumer of pulses, cultivating over an area of approximately 27.51 million hectares with an annual production of 24.25 million tonnes and productivity of 881 kg ha^{-1} (1). Among the various pulse crops, greengram (*Vigna radiata*) holds special importance. In India, greengram is cultivated over 5.2 million hectares, yielding 3.1 million tonnes with a productivity of 598 kg ha^{-1} (2). Greengram is highly nutritious, containing 25 % high-quality protein and is commonly consumed as dhal or whole grains, which are highly digestible.

Despite significant nutritional benefits, achieving higher productivity in greengram cultivation poses numerous challenges for farmers. The challenges are attributed to physiological, biochemical and inherent crop factors. Physiological constraints such as inefficient partitioning of assimilates, poor pod setting, excessive flower abscission and nutrient deficiencies during critical growth stages limit greengram yield. In addition, inherent factors *viz.* poor seed germination, indeterminate growth, less efficient C_3 photosynthetic mechanism, reduced nodule activity and lower sink activity further lower the crop productivity (3). One of the most common and critical factors affecting the productivity of greengram and other pulse crops is flower shedding caused by abscission.

Flower shedding in pulses is a major concern, as each flower has the potential to produce multiple seeds, offering higher yield potential compared to cereals. However, abscission is a major factor contributing to flower shedding. It is triggered by several factors, including limited photosynthesis, hormonal imbalances, nitrogen deficiency, reduced light intensity, humidity, soil and water conditions and genetic factors (4). Abscission follows a progression involving the formation of an abscission zone, activation of detachment signals, organ shedding and differentiation of a protective layer (5).

Flower shedding in greengram is regulated by a complex interplay of physiological and hormonal mechanisms, with auxins and cytokinins having significant

effects. In greengram, the process of flower shedding begins in the specialized abscission zone located at the base of the flower. The region is sensitive to hormonal signals that control the breakdown of cell walls and subsequent separation. Auxins are produced in the developing flower and surrounding tissues and inhibit abscission by maintaining the structural integrity of the abscission zone (6, 7). High auxin levels protect the cells in the zone from degradation, thus preventing premature shedding. As the flower or fruit matures or when the plant experiences environmental stress, auxin levels start to decline, which triggers the activation of other hormones, particularly ethylene, that promote cell wall breakdown and lead to flower shedding. On the other hand, cytokinins act antagonistically to ethylene and play a crucial role in delaying abscission. It helps the flowers remain attached to the plant by promoting cell division and maintaining the integrity of the cell walls in the abscission zone (8, 9). The environmental factors such as water stress, temperature fluctuations and nutrient availability can alter the hormonal balance in greengram, accelerating or delaying the shedding process depending on the plant's adaptive responses.

Controlling flower shedding is a critical area of research in agriculture and horticulture, as it directly impacts both the quantity and quality of crop yield. Strategies to address such problems, include breeding genetic lines with improved flower retention and applying plant growth regulators during flowering stages. Foliar application of plant growth regulators, such as auxin and cytokinin offer a practical and effective solution to mitigate flower shedding, enhance pod retention and ultimately improve the crop yield (10-12). In recent years, nanoformulations of plant growth regulators have emerged as an innovative approach to further enhance their efficiency. Nanoemulsions, characterized by nanoscale droplets of oil-in-water systems, provide a highly effective delivery system for growth regulators (13). Due to unique properties of nanoformulation, including larger surface area, improved wettability and longer retention on plant surfaces, facilitate better uptake by plants even at lower application rates (14). With this study background, the study focuses on synthesizing and characterizing nanoemulsions of auxin and zeatin and evaluating their effects on reducing flower shedding and improving greengram productivity. The research seeks to address the critical issue of flower shedding in greengram cultivation and to develop a sustainable approach to enhance crop productivity, ensuring its continued importance in global agriculture.

Materials and Methods

Laboratory study

Synthesis of naphthalene acetic acid (NAA) nanoemulsion

1-Naphthalene acetic acid (NAA) with 95 % purity was dissolved in different concentrations of ethanol *viz*., 40, 50 and 60 % to optimize the concentration for aqueous phase based on the earlier studies. Similarly, the oil phase was prepared using paraffin oil, tween 80 and ethanol. Tween 80 was used as a non-ionic surfactant and ethanol as a co-surfactant. Different ratio of paraffin oil, tween 80 and ethanol such as 1:5:4, 1:6:3 and 1:4:5 was tested. Nanoemulsion of NAA was prepared by adding the oil phase slowly in the aqueous phase and kept in a magnetic stirrer (Lab Quest MHPS 350) at 600 rpm at 50 ˚C. The resultant solution was subjected to high-pressure homogenization and ultrasonication to obtain desired particle size and polydispersity index. High-pressure homogenization and ultrasonication methods are both commonly used for reducing particle size and controlling the polydispersity index, which is a measure of the distribution of particle sizes in a suspension. High-pressure homogenization is a mechanical process used to reduce the particle size of a liquid or suspension by forcing it through a small nozzle under high pressure. Ultrasonication employs high-frequency sound waves (ultrasound) to agitate a liquid or suspension, promoting the dispersion and breakup of particles. Nanoemulsion with lesser particle size and polydispersity index was selected for further characterization and synthesized for field study.

Synthesis of zeatin nanoemulsion

Zeatin mixed isomer with 95 % purity was dissolved in different concentrations of DMSO such as 0.25, 0.50, 0.75 and 1 % for preparation of the aqueous phase. The oil phase containing paraffin oil, tween 80 and Dimethyl Sulfoxide (DMSO) in the ratio of 1:5:4 and 1:4:5 was tested. Tween 80 acts as a non-ionic surfactant and DMSO as a co-surfactant. Nanoemulsion of zeatin was synthesized by adding the oil phase slowly in an aqueous phase kept in a magnetic stirrer at 600 rpm at 50 ˚C. The resultant solution was subjected to ultrasonication. Based on the particle size and polydispersity index, nanoemulsion was selected for further characterization and synthesized to evaluate the effect on reducing flower shedding in greengram.

Characterization of nanoformulations

Particle size: Particle size analyser (Horiba SZ-100) was used to determine the particle size and polydispersity index of the nanoemulsion. A sample for particle size estimation was prepared by dissolving 1 mL of the nanoemulsion in 10 mL of distilled water. The solution is then thoroughly mixed using a vortex (BR-2000 Vortexer) to ensure uniform dispersion of the particles throughout the solution and to prevent any aggregation that affects the accuracy of the measurement. The prepared sample was transferred into a cuvette for determination of particle size and polydispersity index through dynamic light scattering at a scattering angle of 173˚, a standard angle that provides optimal scattering conditions for measuring particle sizes in the nano range.

Fourier transform infrared spectroscopy (FTIR): Fourier transform infrared spectroscopy (Jasco FT/IR-6800) is a technique used to identify the different functional groups in a sample. FTIR spectrum was recorded in wavenumber between 4000 and 400 cm^{-1}. Nanoemulsion and active ingredients of both auxin and zeatin were used for the identification of corresponding functional groups in nanoemulsions.

Field assessment

The field experiment was carried out at Field No. NA 24, Agricultural Research Station, Bhavanisagar, Erode. The experimental field is located at 11˚29'N latitude and 77˚80'E longitude and belongs to the western agroclimatic zone of Tamil Nadu. The soil of the experimental field was red sandy loam. The field trial was carried out during the *Kharif* season of 2021. The seed of greengram *var.* CO 8 was used for the experiment.

The experiment was laid out in a randomized block design (RBD) and replicated thrice. The seeds were sown with a spacing of 30 cm × 10 cm. The nanoemulsions were evaluated at various doses and compared with both control and recommended doses of conventional formulations of auxin and zeatin to assess their effectiveness in reducing flower shedding. The treatments are Control (T_1), Conventional formulation of NAA at 40 ppm (T_2), Nanoemulsion of NAA at 20 ppm (T_3) and Nanoemulsion of NAA at 30 ppm (T_4), Nanoemulsion of NAA at 40 ppm (T_5), Conventional formulation of Zeatin at 5 ppm (T_6), Nanoemulsion of Zeatin at 2 ppm (T_7), Nanoemulsion of Zeatin at 5 ppm (T_8). The foliar spray of growth regulators was carried out at 50 % flowering and 10 days after the first spray. The recommended fertilizer schedule of 25: 50: 25 kg ha^{-1} was applied as basal. Nitrogen, phosphorus and potassium were supplied with fertilizers such as urea, DAP and murate of potash, respectively.

Growth parameters

Plant height

In each plot, five plants in the net plot were randomly selected and tagged. Plant height was measured in the tagged plants at successive intervals of 15 days from the 15th day after sowing. It was expressed in cm per plant.

Leaf area index (LAI)

The leaf area index (LAI) determines the assimilating area and growth. LAI was estimated by using the following formula (15). Leaf area was measured using a leaf area meter (LI-3100C) and expressed in cm^2.

$$LAI = \frac{\text{Leaf area}}{\text{Ground area}} \qquad \text{(Eqn. 1)}$$

Total dry matter production

Dry matter produced by plants at each growth stage was estimated at 30, 45 and 60 days after sowing (DAS). The plant was uprooted and shade-dried. Subsequently, it was kept in a hot air oven at 65 ˚C until the successive weight of the plant remained unchanged.

Crop growth rate (CGR)

The crop growth rate estimates the rate of growth per unit area and is expressed in g m^{-2} day^{-1}. It was estimated using the following formula (15).

$$CGR = \frac{W_2 - W_1}{P(t_2 - t_1)} \qquad \text{(Eqn. 2)}$$

where,

W_1 = dry weight of plants per unit area at time t_1

W_2 = dry weight of plants per unit area at time t_2

P = plant spacing in m^2

Physiological parameters

Chlorophyll index

The soil plant analysis development (SPAD) meter was used to measure the relative greenness of the leaf. The readings were recorded at 10 and 20 days after the spray of growth regulators and before harvest.

Soluble protein

The fresh leaf sample was collected from the selected plants from individual plot to quantify the soluble protein. Soluble protein was assessed before and after the application of growth regulators to compare the effect of foliar spray. It was estimated using Lowry's method in which phosphate buffer was used to extract soluble protein and analysed using a spectrophotometer (Analytik Jena, Specord 210 Plus) at 660 nm (16). It was expressed in mg of soluble protein per gram of fresh leaf sample.

Yield components and yield

Flower shedding percentage

Flower shedding percentage is an important parameter, employed to understand the effect of conventional and nanoemulsion-based auxin and zeatin formulations on arresting flower drop. It is calculated by using below mentioned formula,

$$\text{Flower shedding percentage} = \frac{\text{Number of flowers dropped}}{\text{Total number of flowers formed}} \times 100 \qquad \text{(Eqn. 3)}$$

Pod setting percentage

The pod setting percentage is calculated by using the formula,

$$\text{Pod shedding percentage} = \frac{\text{Total number of pods formed}}{\text{Total number of flowers formed}} \times 100 \qquad \text{(Eqn. 4)}$$

Number of pods per plant

The mature and immature pods were counted in the tagged plant of each plot and the average was worked out.

Total number of seeds per pod

Seeds were separated from the pod and counted using a seed counter.

Seed yield

Pods were harvested when they turned black and seeds became hard. The matured pods in each treatment plot were picked separately. The second picking (71 DAS) was carried out 6 days after the first picking (65 DAS). The harvested pods for each treatment were threshed individually and expressed in kg ha^{-1}.

Haulm yield

After second picking entire plants from each net plot area were uprooted and dried separately in sunshine for 5 days and weighed. It was expressed in kg ha^{-1}.

1000 seed weight

Thousand seed weights were obtained by counting 1000 seeds using a seed counter and weighted.

Harvest index

The harvest index was worked out to assess the efficacy of nanoformulations in terms of improving crop productivity.

$$\text{Harvest Index} = \frac{\text{Economic yield (kg ha}^{-1})}{\text{Biological yield (kg ha}^{-1})} \quad \text{(Eqn. 5)}$$

Statistical analysis

The statistical analysis was carried out using the technique mentioned in a previous study (17). Data of flower shedding percentage and pod setting percentage were transformed using square root transformation and used for statistical analysis. The critical difference at 5 % level of significance was used to determine the significant difference among treatments. Parameters that were not significant among treatments were indicated as NS.

Results and Discussion

Synthesis and characterization of NAA nanoemulsion

The methodology for synthesizing nanoformulated auxin was standardized based on particle size and polydispersity index (Fig. 1). Various concentrations of ethanol (aqueous phase) were used to achieve complete dissolution of NAA and observed that higher ethanol concentration facilitated faster and more efficient dissolution of NAA. However, it was not selected due to its potential to degrade chlorophyll pigments. Conversely, NAA exhibited instability and settled down after a certain period when using 40 % ethanol. Hence, an ethanol concentration of 50 % was determined to be optimal for preparing the aqueous phase, ensuring effective dissolution of NAA without compromising stability or causing adverse effects (Table 1 & 2).

Nanoemulsion with an oil phase ratio of 1:6:3 did not achieve the nano range, while ratio of 1:5:4 ratio with 15 and 30 min of sonication had polydispersity index (PI) of 3.691 and 0.314, respectively, indicating poor stability of the emulsion. Further, adjustment was made with oil phase ratio of 1:4:5 with a sonication period of 30 min producing nanoemulsion with reduced particle size (298.90 nm) and lower PI (0.175). Thus, the optimal nanoemulsion was synthesized using an aqueous phase of NAA dissolved in 50 % ethanol and an oil phase with 1:4:5 ratio of liquid paraffin, Tween 80 and ethanol, processed by ultrasonication for 30 min. The resultant formulation was used to evaluate the effect of nano-NAA on flower shedding.

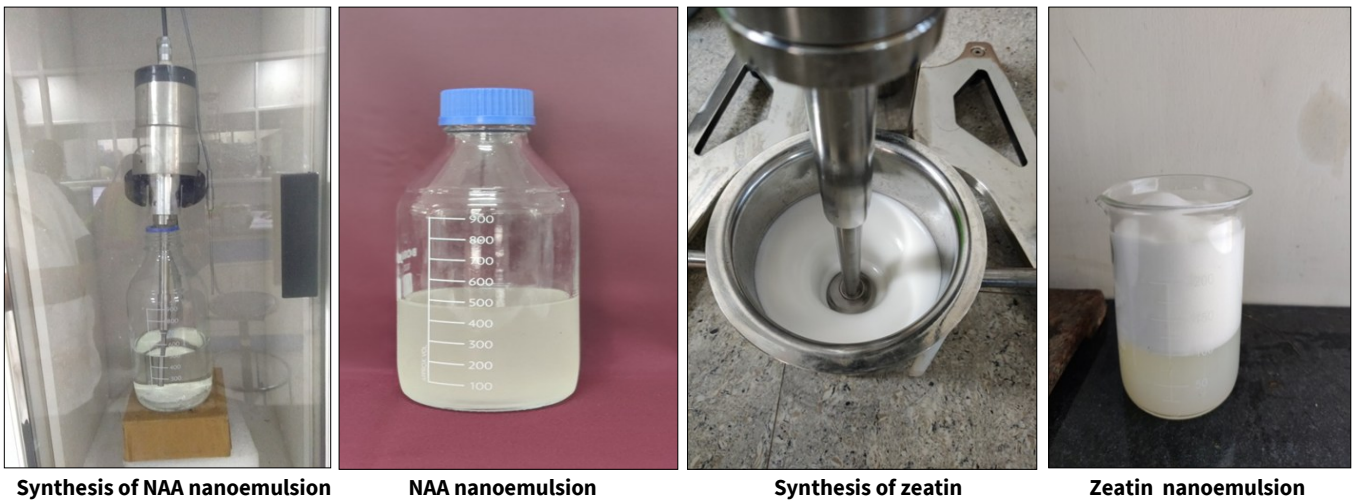
A similar combination of tween 80 and ethanol used in the ratio of 1:1 was proved effective in reducing the surface tension of eucalyptus oil nanoemulsion (18). Further, it was demonstrated that nanoemulsion prepared using a sonicator resulted in better stability and smaller size nanoparticles compared to high-pressure homogenizer. A similar result was

Table 1. Particle size and polydispersity index of NAA nanoemulsion prepared using high-pressure homogenizer

Aqueous phase	Oil phase (Ratio) Liquid paraffin : Tween 80 : 100% Ethanol	No of cycles	Particle size (nm)	Poly dispersity index (PI)
NAA active ingredient dissolved in 50 % ethanol	1:5:4	7	384.9	0.3
	1:6:3	7	838.0	1.1
	1:6:3	10	549.1	0.4

Table 2. Particle size and polydispersity index of NAA nanoemulsion prepared using ultrasonicator

Aqueous phase	Oil phase (Ratio) Liquid paraffin : Tween 80 : 100 % Ethanol	Sonication time (min)	Amplitude (%)	Particle size (nm)	Polydispersity index (PI)
NAA active ingredient dissolved in 50 % ethanol	1:6:3	15	50	Not within nano range	21.000
	1:5:4	15	50	250.0	3.691
	1:5:4	30	50	287.4	0.314
	1:4:5	30	50	298.9	0.175



Synthesis of NAA nanoemulsion | NAA nanoemulsion | Synthesis of zeatin | Zeatin nanoemulsion

Fig. 1. Synthesis of NAA and zeatin nanoemulsion.

observed in the preparation of food grade vitamin D nanoemulsion using an ultrasonicator (19). In addition, a decrease in particle size and polydispersity index was observed with an increase in sonication time in preparation of $LaCoO_3$ powder (20, 21).

The presence of NAA in the nanoemulsion was confirmed through FTIR spectroscopy by identifying characteristic functional group peaks. In the NAA active ingredient, peaks were observed at 3008 cm^{-1} (carboxylic acid group), 2977 cm^{-1} (methyl group), 1739 cm^{-1} (C=O stretching) and 660 cm^{-1} (benzene derivatives) (22). These characteristic peaks with slight shifts, were also present in the NAA nanoemulsion, confirming the presence of NAA in the nanoemulsion (Fig. 2 & 3).

Synthesis and characterization of zeatin nanoemulsion

Dimethyl Sulphoxide (DMSO) was used as a solvent to dissolve the active ingredient, with lower concentrations prioritized to minimize the impact on chlorophyll pigments. The aqueous phases containing 1, 0.75, 0.5 and 0.25 % DMSO were evaluated. The oil phase composed of liquid paraffin, Tween 80 and DMSO was tested in different ratios (1:4:5, 1:5:4, 1:6:3, 1:7:2 and 1:8:1). In the oil phase, DMSO is used as a co-surfactant which has the property to reduce the volume of the surfactant and helps in the formation of stable nanoemulsion (23). Nanoemulsion was synthesized by gradually adding the oil phase into the aqueous phase under magnetic stirring. The nanoemulsion prepared with 1 % DMSO in the aqueous phase and an oil phase ratio of 1:4:5 resulted in high polydispersity index of 5.307 (Table 3). Similarly, 0.75 % DMSO with the same oil phase ratio produced a nanoemulsion with a large particle size (5139.8 nm). Further, 0.5 % DMSO was used with a 1:4:5 oil phase ratio with a sonication period of 5 and 10 min also resulted in higher PI (2.144 and 2.538, respectively). Eventually, nanoemulsions were synthesized using 0.25 % DMSO in the aqueous phase, varying the oil phase ratio with 15 min of sonication. The optimal combination was achieved with an oil phase ratio of 1:4:5, yielding a nanoemulsion with reduced particle size (83.1 nm) and significantly lower PI (1.122). Hence, 0.25 % DMSO as the aqueous phase and an oil phase consisting of liquid paraffin, Tween 80 and DMSO in 1:4:5 ratio, with 15 min of sonication (Fig. 1), was selected for the preparation of zeatin nanoemulsion for further characterization and field study.

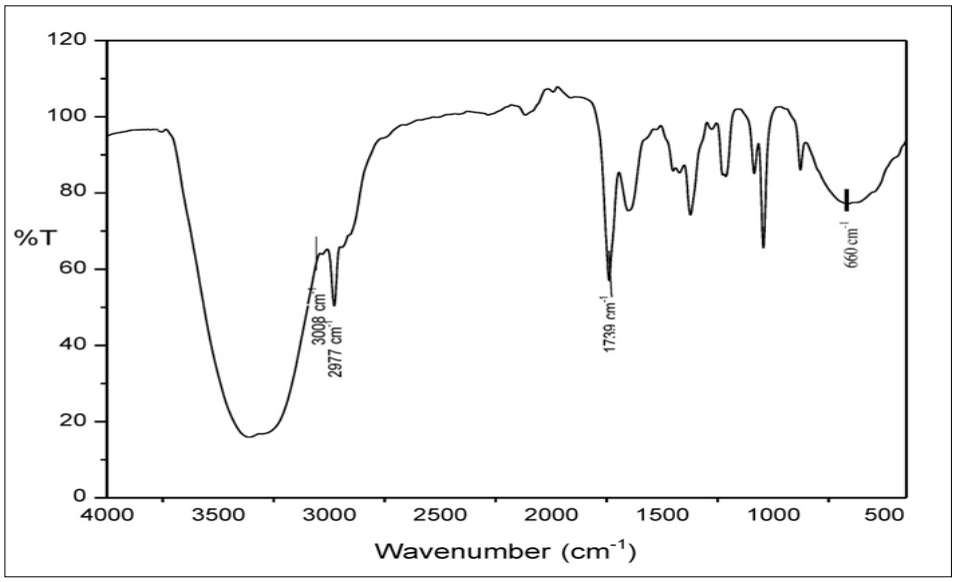


Fig. 2. FTIR spectrum of NAA active ingredient.

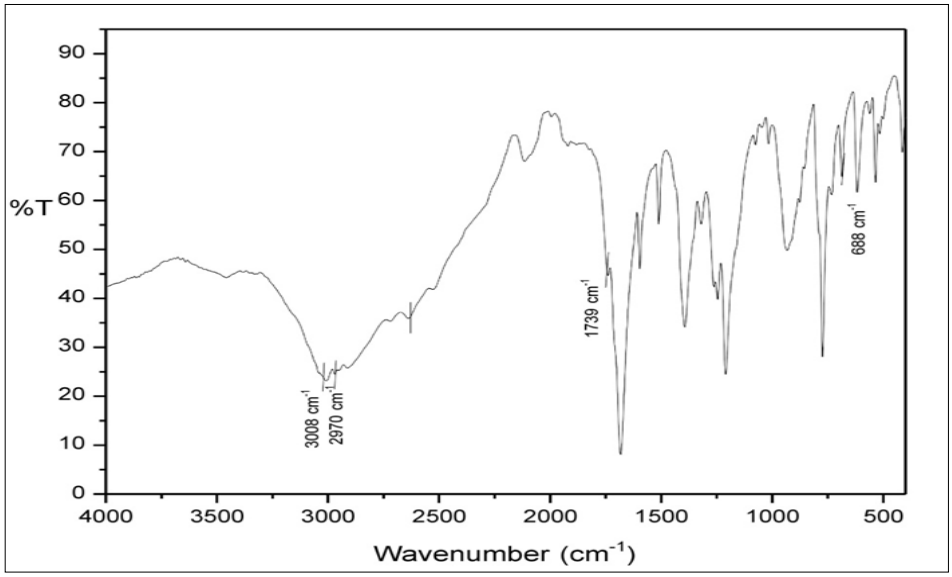


Fig. 3. FTIR spectrum of NAA nanoemulsion.

Table 3. Particle size and polydispersity index of zeatin nanoemulsion using ultrasonicator

Concentration of DMSO (%)	Oil phase (Ratio) Liquid paraffin : Tween 80 : DMSO	Sonication time (min)	Particle size (nm)	Polydispersity index (PI)
1.00	1:4:5	15	435.3	5.307
0.75	1:4:5	15	5139.8	2.853
0.50	1:4:5	5	262.7	2.144
0.50	1:4:5	10	279.2	2.538
0.25	1:4:5	15	83.1	1.122
0.25	1:5:4	15	3142.6	1.400
0.25	1:6:3	15	237.5	1.071
0.25	1:7:2	15	1107.3	2.825
0.25	1:8:1	15	578.1	5.502

The results of FTIR spectra revealed that peaks of functional groups such as N-H stretching at 2975 cm^{-1}, C= C stretching at 1639 cm^{-1}, O-H bending at 1339 cm^{-1}, aromatic amine and C-N stretching at 1088 cm^{-1} were found in FTIR spectra of zeatin active ingredient. The same characteristic peak with slight shifts such as N-H stretching at 2971 cm^{-1}, C= C stretching at 1635 cm^{-1}, O-H bending at 1369 cm^{-1}, aromatic amine and C-N stretching at 1093 cm^{-1} were noted in nanozeatin sample and confirmed the presence of zeatin (Fig. 4 & 5).

Field evaluation of nanoemulsions to arrest the flower shedding

Growth parameters

Higher plant height (13.15 cm) was found in control at 15 DAS (Table 4). However, higher plant height was observed in conventional NAA at 40 ppm (37.27 cm) at 30 DAS and NAA nanoemulsion at 30 ppm at 45 DAS (72.69 cm) and before harvest (72.62 cm). Nanozeatin at 2 ppm was recorded at higher plant height at both 45 DAS and 60 DAS compared to conventional zeatin at 5 ppm, respectively. Auxin and cytokinin are involved in cell division, which results in increased plant height (24). Reduction in plant height with an

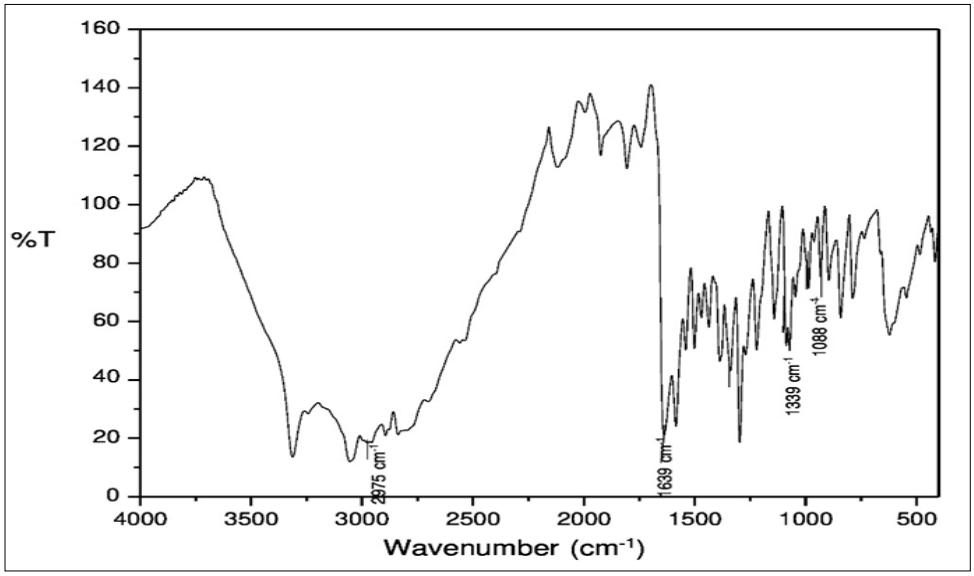


Fig. 4. FTIR spectrum of zeatin active ingredient.

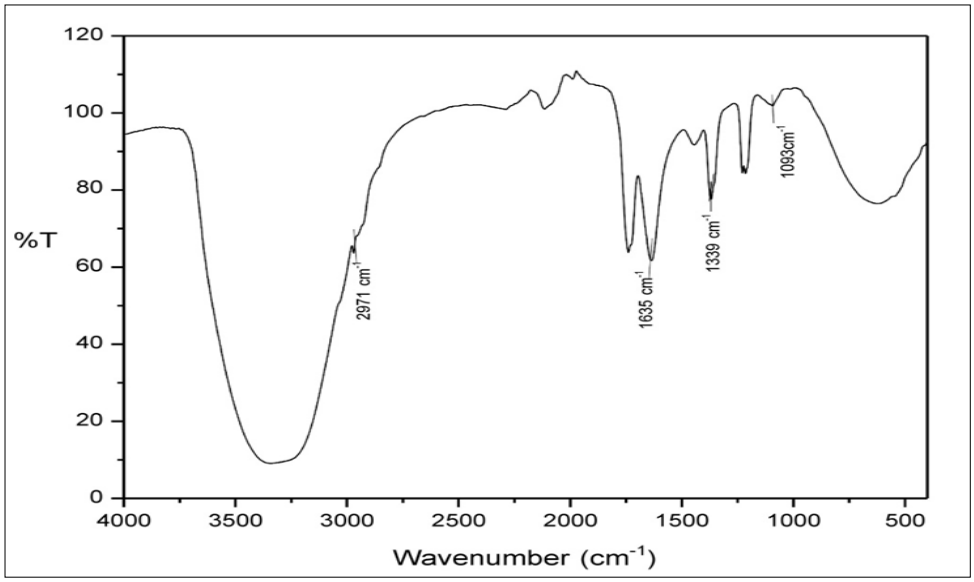


Fig. 5. FTIR spectrum of zeatin nanoemulsion.

Table 4. Effect of NAA and zeatin on plant height (cm plant^{-1}) at different stages of greengram

Treatments	15 DAS	30 DAS	45 DAS	60 DAS
Control (T_1)	13.15	36.65	70.51	68.27
Conventional NAA at 40 ppm (T_2)	12.63	37.27	71.28	70.12
Nanoemulsion of NAA at 20 ppm (T_3)	11.22	36.93	70.73	71.70
Nanoemulsion of NAA at 30 ppm (T_4)	12.84	36.51	72.69	72.62
Nanoemulsion of NAA at 40 ppm (T_5)	11.78	34.45	71.21	69.94
Conventional formulation of Zeatin at 5 ppm (T_6)	11.25	36.44	70.71	71.42
Nanoemulsion of Zeatin at 2 ppm (T_7)	11.41	36.86	71.76	71.44
Nanoemulsion of Zeatin at 5 ppm (T_8)	11.37	37.16	70.59	71.96
SEd	0.96	1.61	1.46	1.58
CD (P=0.05)	NS	NS	NS	NS

increase in concentration of nanoformulated NAA and zeatin was observed however, it was statistically non-significant. The inhibitory effect of cytokinin might be due to the indirect effect of elevated cytokinin on apical dominance (25). Similarly, NAA at above certain concentrations resulted in elevated levels of abscisic acid in the plant system (26).

The leaf area index was maximum in plots applied with nanoemulsion of NAA at 30 ppm (3.62) and found on par with nano zeatin at 2 ppm (3.61) at 45 DAS (Table 5). Further, higher LAI was recorded in NAA nanoemulsion applied at 30 ppm (2.78) and lower in control (1.78) at 60 DAS. It might be due to increased leaf area by foliar application of growth regulators.

In addition, foliar application of NAA nanoemulsion at 30 ppm resulted in higher dry matter accumulation at both 45 DAS (2874 kg ha^{-1}) and 60 DAS (4009 kg ha^{-1}). It was statistically on par with the application of nano zeatin at 2 ppm (2772 kg ha^{-1}) at 45 DAS. The reduction in dry matter production was observed in plots treated with increased concentrations of nano NAA at 40 ppm and nano zeatin at 2 ppm (Fig. 6). Lower dry matter production was noticed in control. The auxin and cytokinin promote cell division and cell elongation which causes increased leaf area and chlorophyll. It eventually leads to an increase in dry matter accumulation. A similar effect of an increase in dry matter production with NAA application was reported in greengram and brinjal at 40 ppm (27). In addition, it was noted that foliar spray of kinetin increased the dry matter production in greengram (28).

The crop growth rate was higher in nanoemulsion of NAA at 30 ppm during the entire crop period (Table 6). Further, it was found on par with nanozeatin at 2 ppm and conventional zeatin at 5 ppm at 30-45 DAS. Further, nanoemulsion of NAA at 30 ppm was noticed with a maximum CGR of 7.57 g m^{-2} day^{-1} among other treatments at 45-60 DAS. In addition, nano NAA at 30 ppm and conventional zeatin at 5 ppm were found statistically similar to each other.

A lower crop growth rate was observed in the control. The increased crop growth rate was observed following the application of NAA and zeatin, highlighting the positive effects of application of growth regulators on crop growth and development. Specifically, higher levels of the cytokinin trans-zeatin riboside exhibited a positive correlation with leaf expansion. An elevated concentration of cytokinins in the leaves enhances photosynthetic activity, which in turn increases sugar production, facilitating improved leaf expansion and plant growth (29). This mechanism likely contributed to the increase in crop growth rate due to the application of zeatin. Moreover, nanoformulations significantly enhance the bioavailability and efficacy of growth regulators such as auxin and zeatin by improving their stability, solubility and absorption. It resulted in nanoformulations were found superior to conventional formulations, ensuring more effective delivery and better plant response.

Table 5. Effect of NAA and zeatin on leaf area index at different stages of greengram

Treatments	30 DAS	45 DAS	60 DAS
Control (T_1)	1.31	2.52	1.78
Conventional NAA at 40 ppm (T_2)	1.19	3.09	2.38
Nanoemulsion of NAA at 20 ppm (T_3)	1.26	3.11	2.59
Nanoemulsion of NAA at 30 ppm (T_4)	1.22	3.62	2.78
Nanoemulsion of NAA at 40 ppm (T_5)	1.36	2.87	2.28
Conventional formulation of Zeatin at 5 ppm (T_6)	1.20	3.43	2.77
Nanoemulsion of Zeatin at 2 ppm (T_7)	1.25	3.61	2.70
Nanoemulsion of Zeatin at 5 ppm (T_8)	1.26	2.73	2.26
SEd	0.02	0.06	0.09
CD (P=0.05)	0.05	0.13	0.19

Table 6. Effect of NAA and zeatin on crop growth rate (g m^{-2} day^{-1}) of greengram

Treatments	30 DAS–45 DAS	45 DAS–60DAS
Control (T_1)	8.77	2.33
Conventional NAA at 40 ppm (T_2)	12.03	4.23
Nanoemulsion of NAA at 20 ppm (T_3)	12.16	5.78
Nanoemulsion of NAA at 30 ppm (T_4)	14.83	7.57
Nanoemulsion of NAA at 40 ppm (T_5)	10.62	5.06
Conventional formulation of zeatin at 5 ppm (T_6)	13.83	7.35
Nanoemulsion of Zeatin at 2 ppm (T_7)	14.06	4.86
Nanoemulsion of Zeatin at 5 ppm (T_8)	10.17	5.17
SEd	0.47	0.42
CD (P=0.05)	1.00	0.91

Physiological parameters

The chlorophyll index recorded before foliar spraying did not show a significant difference among treatments (Table 7). However, a significant difference was observed after the foliar spray of growth regulators. Nanoemulsion NAA applied at 30 ppm recorded a higher chlorophyll index of 50.79, 36.45 and 23.57 at 10 days after spraying, 20 days after spraying and before harvest respectively. NAA prevents the photooxidation of chlorophyll molecules which is the primary reason for the increase in chlorophyll pigment (30). IAA which indirectly enhanced chlorophyll synthesis in maize by increasing magnesium content (31). Magnesium is a fundamental element required for the synthesis of chlorophyll and down-signaling chlorophyll degradation. Exogenous application of NAA increased the chlorophyll content in various crops such as wax apple, brinjal, soybean, pearl millet and cowpea (27, 32-36). In addition, it was found that cytokinin has the potential to increase chloroplast number per cell in plants (37). A similar finding of an increase in chlorophyll content due to a foliar spray of kinetin was reported in mungbean and *Cathranthus roseus* (38, 39).

Similarly, application of growth regulators increased the soluble protein level; however, there was no significant difference among treatments (Table 8). Nanoemulsion of zeatin applied at 2 ppm registered higher soluble protein content at 5 days after spraying (29.36 mg g^{-1}) and 10 days after spraying (27.30 mg g^{-1}), which was followed by nano NAA at 30 ppm. The increase in soluble protein (22.15, 24.26 and 25.69 mg g^{-1}) due to increased concentration of NAA (25, 50 and 75 ppm) was documented in greengram cv. GM-4 (40). Further, cytokinin is involved either directly in protein synthesis or indirectly by increasing RNA synthesis (41, 42). It inhibits the degradation of protein and sustains the protein level in the plant system which is attributed to increased soluble protein content (43).

Yield parameters

Flower shedding percentage was reduced due to the application of growth regulators and however, increased at higher doses of nanoformulations (Fig. 7). It was observed that flower shedding was minimal with foliar spray of NAA nanoemulsion at 30 ppm (20.7 %). It was on par with plots treated with conventional zeatin at 5 ppm (21.1 %) and nanozeatin at 2 ppm (23.7 %). A higher flower shedding percentage (52 %) was recorded in the control plot followed by foliar spray of nanozeatin at 5 ppm (37.6 %). Exogenous application of synthetic auxin and cytokinin alters the physiological process and reduces flower shedding. The reduced flower shedding percentage by NAA application was reported in broad beans (44). Similarly, an increase in cytokinin content in *Vicia faba* at flowering arrested the flower shedding (45).

A lower pod setting percentage (42.4 %) was registered in the control plot. Foliar application of growth regulators increased the pod setting percentage by reducing flower shedding in greengram (Fig. 7). A higher pod setting percentage (80.7 %) was noticed in the plot treated with nanoemulsion of NAA at 30ppm and performed better over other treatments. Increased pod setting was due to a reduction in the dropping of flowers in greengram. The reduced pod setting (39.73 pods $plant^{-1}$) was noted in the control plot. Foliar spray of growth regulators promoted pod setting in greengram and was influenced by dosage and formulation of NAA and zeatin. A higher number of pods resulted from NAA nanoemulsion sprayed at 30 ppm (93.25 pods $plant^{-1}$) followed by plots applied with conventional zeatin formulation at 5 ppm (90.77 pods $plant^{-1}$) and nano zeatin applied at 2 ppm (90.75 pods $plant^{-1}$).

Table 7. Chlorophyll index of greengram before and after foliar spray

Treatments	Chlorophyll index			
	Before spraying (30 DAS)	10 Days after spraying	20 Days after spraying	Before harvest
Control (T_1)	41.51	40.53	23.44	20.96
Conventional NAA at 40 ppm (T_2)	41.65	50.52	34.87	22.99
Nanoemulsion of NAA at 20 ppm (T_3)	42.29	48.19	35.07	23.57
Nanoemulsion of NAA at 30 ppm (T_4)	42.00	50.79	36.45	23.57
Nanoemulsion of NAA at 40 ppm (T_5)	40.95	49.28	33.98	22.77
Conventional formulation of Zeatin at 5 ppm (T_6)	42.65	45.27	26.79	22.79
Nanoemulsion of Zeatin at 2 ppm (T_7)	40.37	49.99	25.47	22.48
Nanoemulsion of Zeatin at 5 ppm (T_8)	39.48	49.79	24.7	21.22
SEd	2.29	2.11	0.33	0.48
CD (P=0.05)	NS	4.52	0.78	1.13

Table 8. Effect of NAA and zeatin on soluble protein (mg g^{-1}) of greengram before and after foliar spray

Treatments	Before spraying	5 days after spraying	10 days after spraying
Control (T_1)	12.221	19.18	20.06
Conventional NAA at 40 ppm (T_2)	14.403	22.92	26.81
Nanoemulsion of NAA at 20 ppm (T_3)	12.221	19.80	21.96
Nanoemulsion of NAA at 30 ppm (T_4)	12.829	26.29	26.83
Nanoemulsion of NAA at 40 ppm (T_5)	13.954	21.29	24.98
Conventional formulation of Zeatin at 5 ppm (T_6)	12.082	25.65	23.30
Nanoemulsion of Zeatin at 2 ppm (T_7)	12.063	29.36	27.30
Nanoemulsion of Zeatin at 5 ppm (T_8)	10.659	24.19	23.71
SEd	2.75	4.74	4.09
CD (P=0.05)	NS	NS	NS

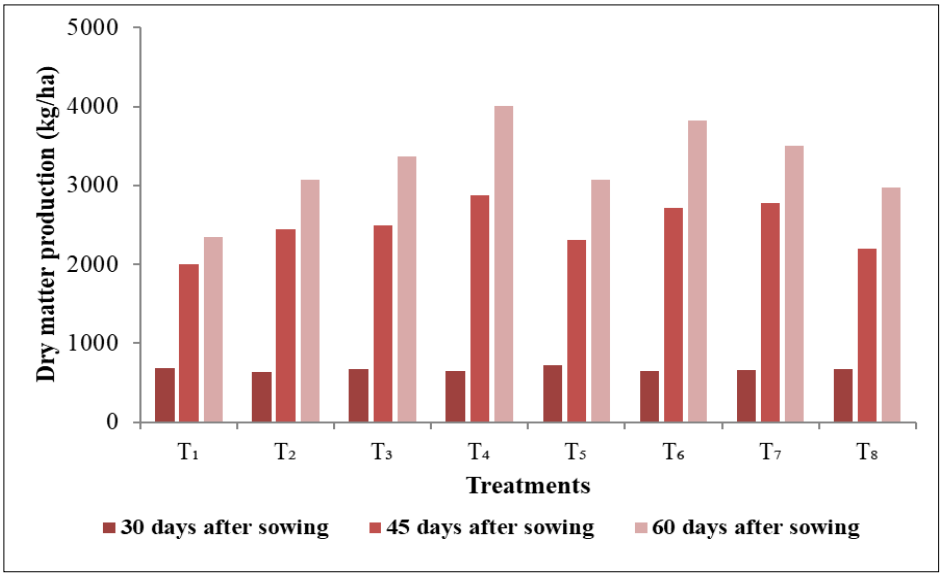


Fig. 6. Effect of foliar spray of NAA and zeatin on total dry matter accumulation of greengram**.**

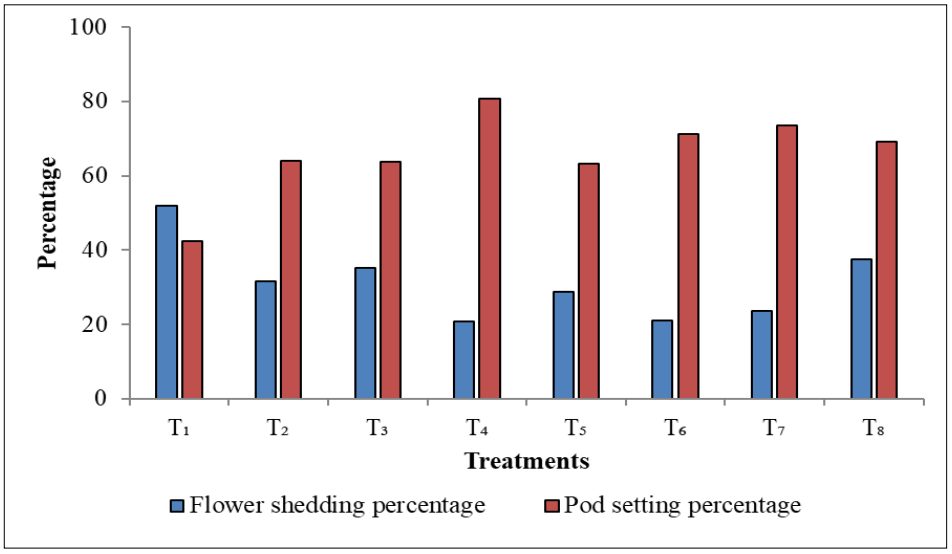


Fig. 7. Influence of foliar application of NAA and zeatin on flower shedding and pod setting percentage.

Further, plots sprayed with plant growth regulators were registered with increased pod number per plant over control (Table 9). Flower shedding reduction was the primary factor responsible for the increased pod number in plots sprayed with NAA and zeatin. Auxin present in the shoot tips is involved in the regulation of the source-sink relationship by mediating the flow of metabolites (46). It might be the reason for the increase in pod number in NAA sprayed plots. A similar result of increased pods per plant in mungbean and chickpea was noticed due to NAA spray (47, 48). On the other hand, it was found that cytokinin increases sink strength by increasing the expression of extracellular invertases (37). It was attributed to the increase of pod number in cytokinin-treated plots. A smaller number of seeds pod^{-1} was recorded in control. More number of seeds pod^{-1} was observed in plots sprayed with nanoemulsion of NAA at 30 ppm. The increased number of seeds pod^{-1} by the application of NAA and zeatin was due to increased leaf area and dry matter accumulation which attributed to the synthesis of photo-assimilates at higher levels (49). Thousand seed weight was not significantly influenced by treatments. Application of nanoemulsion of NAA at 30 ppm had higher test weight (29.91 g) over other treatments.

Higher seed yield (1096 kg ha^{-1}) was obtained in plots applied with NAA nanoemulsion at 30 ppm (Fig. 8). The treatment was statistically on par with the foliar application of conventional zeatin at 5 ppm (1036 kg ha^{-1}) and nano zeatin at 2 ppm (954 kg ha^{-1}). Seed yield was decreased at higher doses of nanoemulsion of NAA and zeatin. Similarly, haulm yield was also significantly influenced by treatments. A foliar spray of nano NAA at 30 ppm produced a higher haulm yield followed by conventional zeatin at 5 ppm. Control recorded lower seed and haulm yield. Lower flower shedding percentage, higher pod setting percentage and greater number of seeds per pod are favoured for higher yield. The increase of haulm yield in treated plots compared to control might be due to an increase in dry matter production continuously after foliar spray. The harvest index was not significantly influenced by treatments. Plots applied with nanoemulsion of NAA at 30 ppm, nano NAA at 40 ppm and conventional zeatin at 5 ppm recorded a higher harvest index (0.27). However, control and nano zeatin at 5 ppm had a lower harvest index of 0.25.

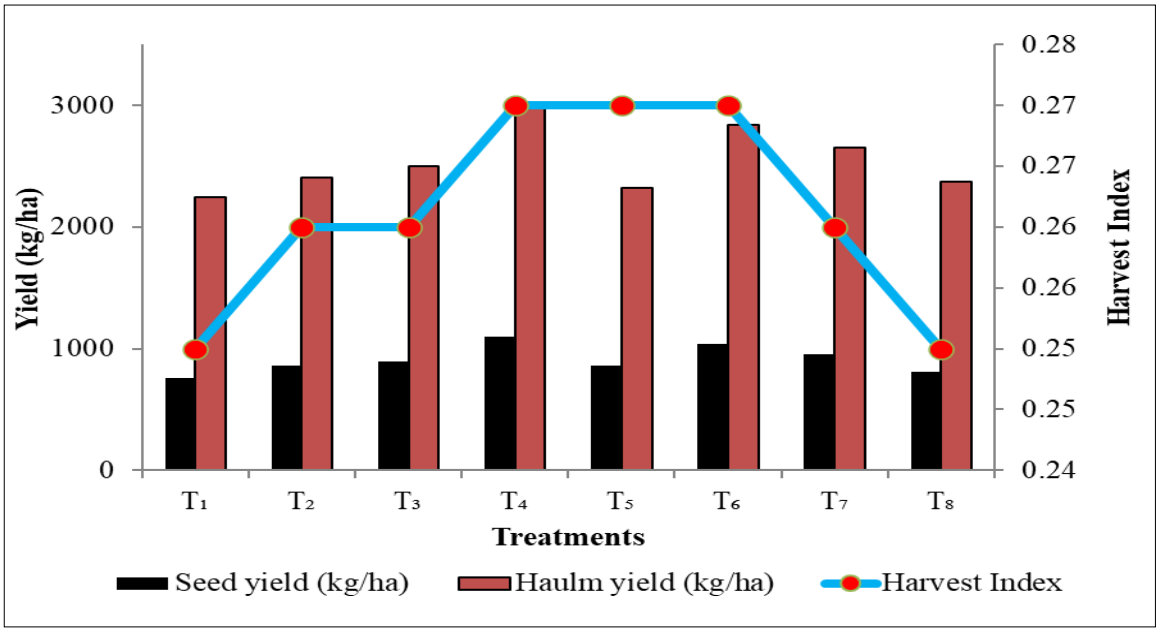


Fig. 8. Effect of foliar spray of NAA and zeatin on seed yield, haulm yield and harvest index of greengram.

Table 9. Effect of foliar application of NAA and zeatin on yield attributes of greengram

Treatments	Total number of pods plant^{-1}	Total no of seeds pod^{-1}	1000 seed weight (g)
Control (T_1)	39.73	7.94	26.80
Conventional NAA at 40 ppm (T_2)	79.43	9.78	28.85
Nanoemulsion of NAA at 20 ppm (T_3)	58.85	9.41	29.28
Nanoemulsion of NAA at 30 ppm (T_4)	93.25	11.00	29.91
Nanoemulsion of NAA at 40 ppm (T_5)	69.79	9.09	27.40
Conventional formulation of Zeatin at 5 ppm (T_6)	90.77	10.66	28.58
Nanoemulsion of Zeatin at 2 ppm (T_7)	90.75	9.73	28.18
Nanoemulsion of Zeatin at 5 ppm (T_8)	66.70	9.57	28.44
SEd	3.36	0.72	1.26
CD (P=0.05)	7.20	1.54	NS

Conclusion

From the study, nanoemulsions of NAA and zeatin were synthesized and standardized. The NAA nanoemulsion was prepared using 50 % ethanol as the aqueous phase and a combination of liquid paraffin, Tween 80 and ethanol in the ratio of 1:4:5 as oil phase, with sonication for 30 min. Similarly, zeatin nanoemulsion was formulated using 0.25 % DMSO as the aqueous phase and an oil phase comprising liquid paraffin, Tween 80 and 0.25 % DMSO in 1:4:5 ratio. Both nanoemulsions demonstrated reduced particle size and enhanced stability, making them suitable for agricultural applications. The field application of nanoformulated NAA and zeatin at 50 % flowering and 10 days after the first foliar spray significantly improved growth, physiological traits and yield. Among the treatments, the nanoemulsion of NAA at 30 ppm was found as the most effective, achieving the highest pod setting percentage and seed yield (1096 kg ha^{-1}). The findings underscore the potential of nanoformulations as a sustainable and efficient approach to enhance crop productivity. Further, research should focus on assessing the long-term impacts of nanoformulations on soil health and microbial ecosystems, as well as exploring their effects on other pulse crops. Additionally, optimizing formulation parameters and evaluating their environmental impact and potential for combining with other agrochemicals, would enhance the sustainability and efficiency of nanoemulsion in agriculture.

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Authors' contributions

KP did the conceptualization, data curation, formal analysis, visualization and writing of the original draft. PP contributed to the writing of the original draft. MS helped in conceptualization, methodology, supervision and writing, review and editing.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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