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Post Flowering Management Using Plant Growth Regulators in Greengram (*Vigna radiata* L.)

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Authors' contributions

This work has been carried out with guidelines of all authors. All authors read and approved the manuscript.

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ABSTRACT

Aim: The study was conducted to evaluate the effect of Auxin and Zeatin-based nanoformulations in post-flowering management in greengram.
Place and Duration of Study: Nanoformulations were standardized at the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore and a field trial was carried out at Agricultural Research Station, Bhavanisagar.
Methodology: Nanoformulations were standardized by oil in water nanoemulsion technique and effect on crop is observed by foliar spray of nanoformulations during the flowering stage of crop growth.

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Results: Increased chlorophyll content due to NAA and Zeatin and extended retention of chlorophyll in Auxin-based treatments has been recorded. Hence the crop can produce more assimilates leading to increased seed yield. Zeatin spray also recorded increased chlorophyll, mature pods, number of seeds pod^{-1}, seed weight, pod setting percentage. Such an increase of yield attributes was mainly due to decreased flower shedding percentage.

Conclusion: Growth regulators such as Auxin and Zeatin exhibited a significant role in increasing plant chlorophyll content and yield of greengram. Among treatments, Nanoemulsion of NAA @ 30ppm recorded a higher number of mature pods, seed yield, seed weight and lower flower shedding percentage which is concluded as the best treatment.

Keywords: Greengram; naphthalene acetic acid; zeatin; post flowering management; chlorophyll; yield attributes.

1. INTRODUCTION

Greengram (*Vigna radiata* L.) is a versatile crop that is the third most important pulse crop in India. Greengram is a good source of protein. The safe dietary reference intake of protein for an average human who weighs 75 kg is 56 grams per day, whereas the per capital availability of legumes in India is 43.8 grams per day which is much lower than the protein requirement of adults. During 2020-21, Greengram is cultivated in an average area of 5.13 million hectare, production of 3.09 million tonners and productivity of 601 kg ha^{-1} [1]. Moreover, average yield gap of 48.73 percent was observed in 2019 which indicates a need of increase in production of greengram [2]. Possible ways to increase in the pulse production in India is by increasing area under pulses and raising the productivity of pulses per unit area of land [3]. Important way to increase pulse production is by maintaining crop during post flowering stage, as excess flower abscission lead to poor pod setting percentage eventhough the crop having excessive vegetative growth pattern and profuse flowering [4]. Flower as well as pod shedding is common feature in this crop which is reflected in sink realization. Flower shedding is due to physiological, biochemical and also certain inherent factors associated with the crop. The physiological factors such as inefficient partitioning of assimilates, poor pod setting, excessive flower abscission and deficiency of nutrients during the critical stages of crop growth were found to be important yield barriers for greengram [5].

Post flowering management in pulses is an important practice to increase productivity. It is the activity carried out to reduce flower shedding, increase pod setting and thereby to increase yield foliar spray of nutrients and plant growth regulators has been carried out during 50% flowering and peak flowering stages. Growth regulators improves the physiological efficiency such as photosynthetic ability and enhances effective partitioning of accumulates from source and sink [6] plays a significant role in raising the productivity of the crop [7]. Foliar feeding of growth regulators is the most effective and economical way to improve plant nutrient need [8].

Auxins are plant hormones controlling plant growth and development at different environmental conditions [9]. Naphthalene Acetic Acid (NAA) is an aromatic acid which belongs to synthetic plant hormone of auxin family. It acts as a somatotrophin like growth regulators in plant system by stimulating cell division and elongation membrane permeability, RNA synthesis, water uptake and in physiological processes such as prevention of fruit drop and bud sprouting, increase pod numbers, delays senescence, increase flower induction, fruit setting and also ultimately increases yield [10]. Naturally occurring auxins are Indole Acetic Acid (IAA), Indole butyric Acid (IBA), Phenyl Acetic Acid (PAA), etc. Synthetic auxins are Napthalene Acetic Acid (NAA), Indole butyric acid (IBA), 2-methyl-4-chloro-phenoxyaectic acid (MCPA), 2,4-dichloro-phenoxyaectic acid (2,4-D) and 2,4,5-trichloro- phenoxyacetic acid [11].

Cytokinins are adenine derived plant growth hormones that are responsible for various plant metabolisms. Cytokinin delays senescence by delaying chlorophyll breakdown [12] and by reducing the mRNA leading to reduced protein levels of proteases enzyme [13]. Zeatin is the first natural cytokinin isolated from maize [14]. Other than zeatin there are also various other forms of cytokinin such as adenine, kinetin (artificial aromatic cytokinin), 6-benzylamino purine (BA) and benzyladenine (natural aromatic cytokinin), thidiazuron and diphenyl urea (phenyl urea type cytokinin). Zeatin side chain contains a double bond and hydroxy group oriented in a *trans*- or *cis*-configuration representing *trans*-zeatin (tZ) or *cis*-zeatin (cZ), respectively [15]. Exogenous application [16] or increased exogenous production of cytokinins, overexpressing a senescence-associated gene specific promoter for driving the expression of the isopentenyl transferase gene delays senescence [17]. Cytokinin found to increase sink strength in *Arabidopsis thaliana* [18] ultimately lead to higher yield.

Nano formulation is emerging technology for effective delivery mode of active molecules in agriculture due to its special features such as greater surface area, better wettability, and longer retention over the leaf surface facilitating the better uptake in plants. Nanoemulsions are those formed by very small emulsion nanoscale droplets (oil/water system) exhibiting size less than 100 nm [19,20]. Plant growth regulator in the form of nanoemulsion is a new way of approach in post flowering management in greengram.

2. MATERIALS AND METHODS

2.1 Experimental Details

Field experiment was conducted at Agricultural Research Station, Bhavanisagar, Erode located at 11˚ 29' N latitude and 77˚ 80' E longitude to study the effect of Nanoformulated Auxin and Zeatin for reducing flower shedding in greengram. The variety of CO 8 greengram has been sown in spacing of 30x10 cm. Auxin and Zeatin nanoemulsion has been synthesized in department of Nano Science and Technology, TNAU, Coimbatore.

2.2 Treatment Details

T_1 - Control
T_2 - Conventional formulation of NAA @ 40 ppm
T_3 - Nanoemulsion of NAA @ 20 ppm
T_4 - Nanoemulsion of NAA @ 30 ppm
T_5 - Nanoemulsion of NAA @ 40 ppm
T_6 - Conventional formulation of Zeatin @ 5 ppm
T_7 - Nanoemulsion of Zeatin @ 2 ppm
T_8 - Nanoemulsion of Zeatin @ 5 ppm

Conventional NAA treatment has been imposed by dissolving NAA using 50 % of ethanol and Zeatin has been prepared by dissolving in 0.25 % DMSO. Spraying of conventional and nanoformulated Auxin and Zeatin has been carried out during 50 % flowering and peak flowering stages respectively.

2.3 Observations Recorded

Changes in the chlorophyll content has been recorded by non-destructive method using SPAD chlorophyll meter reading (SCMR) both before and after spraying at 30th, 45th and 60th and 70th DAS. Yield parameters to demarcate the effect of growth regulators have been recorded in five tagged plants from each plot. Numbers of clusters, number of mature, immature pods, number of flowers, total number of seeds/plant are recorded in 5 tagged plants before harvest. Harvest has been carried out on 65 and 75 DAS as 1st picking and 2nd picking respectively to pursue clear idea about effect of growth regulators in yield of greengram. Picking of pods has been carried out and postharvest management practices have been followed.

Flower shedding count has been carried out every 3 days once from the day of spraying from tagged plants regularly. Pod setting percentage has been calculated from total number of pods formed which includes both mature and immature pods and total number of flowers formed which is been as calculated as mentioned above.

Flower shedding percentage (%) =
$$\frac{\text{Number of shed flowers}}{\text{Total number of flowers formed}} \times 100$$

Pod setting percentage (%) =
$$\frac{\text{Total number of pods formed}}{\text{Total number of flowers formed}} \times 100$$

2.4 Statistical Analysis

Data recorded were analysed using AGRES software. Grand mean, Critical difference (CD) at 5% level of probability, and standard error (SE_d) were analysed to find the significant difference between treatments. Parameters that exhibit no significant difference has been mentioned as NS.

3. RESULTS AND DISCUSSION

Chlorophyll pigment recorded before spraying expressed no significant difference whereas chlorophyll content after spraying of NAA and zeatin compared to control (Table 1). Chlorophyll pigment recorded before spraying in control (41.51) and after spraying (40.53) expressed a decreased chlorophyll pigment whereas treatment with auxin and zeatin exhibited increase in chlorophyll pigment which clarifies that auxin and zeatin plays a effective role in increasing chlorophyll content. Among treatments Nanoemulsion of NAA @ 30ppm (50.79) exhibit higher chlorophyll content followed by Nanoemulsion of Zeatin @ 2ppm (49.99) respectively. Similar results were recorded in brinjal [21] and soybean @ 40 ppm NAA [22] and in pearl millet [23] and cowpea [24] @ 20 ppm NAA. Increased chlorophyll content (1.61, 1.83 and 1.83) due to spray of kinetin @ at 0.01, 1.0 and 100 µM compared to control (1.45) was observed during 35 DAS in Mungbean [25]. Similarly, static increase in chlorophyll content with increase in concentration of NAA is recorded in hybrid sorghum [26].

Table 1. Influence of conventional and nanoformulated NAA and Zeatin in SCMR value of greengram

Treatment	SCMR (SPAD chlorophyll meter reading)			
	Before spraying	10 Days after spraying	20 Days after spraying	Before harvest
Control	41.51	40.53	23.44	20.96
Conventional NAA@ 40 ppm	41.65	50.52	34.87	22.99
Nanoemulsion of NAA @ 20ppm	42.29	48.19	36.45	23.57
Nanoemulsion of NAA @ 30ppm	42.00	50.79	35.07	23.17
Nanoemulsion of NAA @ 40ppm	40.95	49.28	33.98	22.77
Conventional formulation of Zeatin @ 5ppm	42.65	45.27	26.79	22.79
Nanoemulsion of Zeatin @ 2ppm	40.37	49.99	25.47	22.48
Nanoemulsion of Zeatin @ 5ppm	39.48	49.79	24.7	21.22
Grand mean	41.36	48.04	30.20	22.44
SE_d	2.29	2.11	0.33	0.48
CD (0.05)	NS	4.52	0.78	1.13

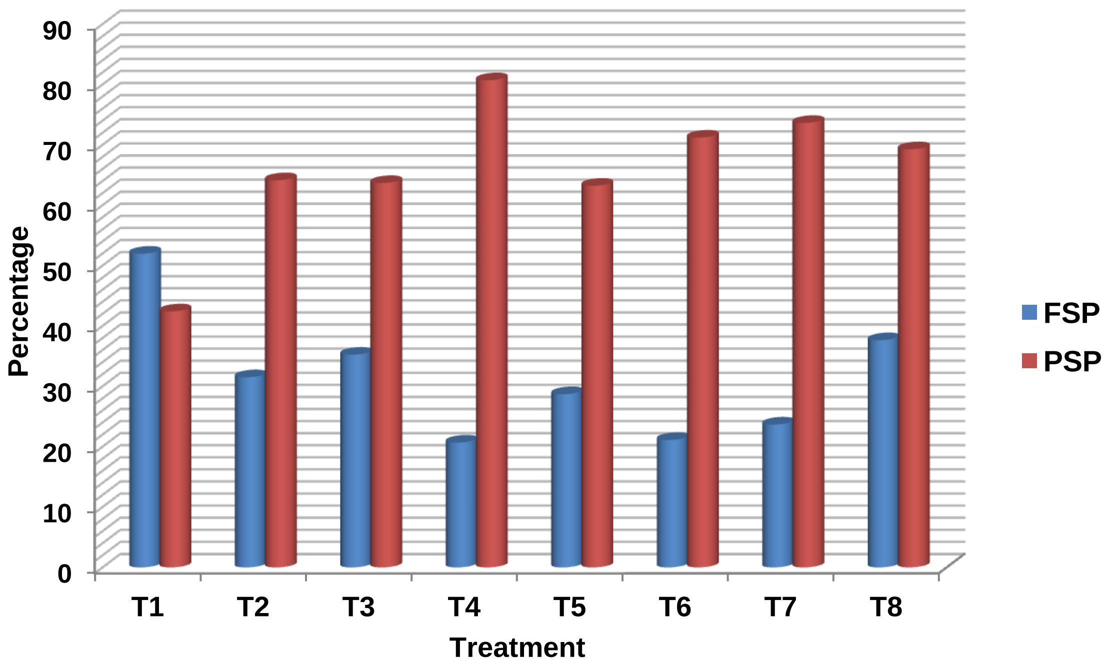

Fig. 1. Flower Shedding Percentage (FSP) and Pod Setting Percentage (PSP) of greengram after impose of treatments

Table 2. Influence of conventional and nanoformulated NAA and Zeatin based nanoemulsion in yield attributes of greengram

Treatment	No of clusters	Number of immature pods	Number of mature pods	Total number of pods formed	No of dropped flowers
Control	17.13	8.49	31.76	39.73	49.38
Conventional NAA@ 40 ppm	23.00	28.33	48.46	79.43	39.07
Nanoemulsion of NAA @ 20ppm	20.40	11.81	50.26	58.85	34.95
Nanoemulsion of NAA @ 30ppm	22.47	24.36	73.61	93.25	27.18
Nanoemulsion of NAA @ 40ppm	20.40	17.48	55.84	69.79	32.54
Conventional formulation of Zeatin @ 5ppm	20.67	31.90	66.13	90.77	28.53
Nanoemulsion of Zeatin @ 2ppm	21.40	37.53	52.68	90.75	27.37
Nanoemulsion of Zeatin @ 5ppm	21.73	17.63	49.33	66.70	32.38
Grand mean	20.90	22.19	53.51	73.66	33.92
SE_d	1.62	3.15	4.00	3.36	2.02
CD (0.05)	NS	6.76	8.58	7.20	4.32

Treatment	Flower shedding percentage (%)	Pod setting percentage (%)	Total no of seeds plant^{-1}	Seed weight plant^{-1} (gram)
Control	51.97	42.41	396.27	10.75
Conventional NAA@ 40 ppm	31.51	64.14	518.16	14.35
Nanoemulsion of NAA @ 20ppm	35.24	63.68	485.00	15.84
Nanoemulsion of NAA @ 30ppm	20.68	80.70	591.12	17.95
Nanoemulsion of NAA @ 40ppm	28.71	63.23	504.97	15.86
Conventional formulation of Zeatin @ 5ppm	21.11	71.20	526.56	16.92
Nanoemulsion of Zeatin @ 2ppm	23.67	73.63	520.24	14.73
Nanoemulsion of Zeatin @ 5ppm	37.64	69.27	485.07	10.64
Grand mean	31.32	66.03	503.42	14.63
SE_d	3.15	2.86	44.64	2.14
CD (0.05)	6.75	6.14	95.76	4.59

Chlorophyll content observed during 20 Days After Spraying also showed significant difference among auxin based treatments and zeatin based treatments. Effect of NAA in retaining chlorophyll content has been extended to a longer period compared to zeatin. Nanoemulsion NAA @ 30 ppm (36.45) found to be best among all other treatment in retaining chlorophyll pigment for long period compared to other treatments. NAA influenced chlorophyll content by preventing photooxidation of chlorophyll molecules [27,28] due to maximum photosynthetic rate and higher leaf area caused by auxin. Similar results were observed in wheat by the effect of IAA @ 40 ppm [29] Maintenance of chlorophyll pigment with the exogenous application of zeatin ribose (ZR) is observed in creeping grass [21]. This clearly indicates that both auxin and zeatin plays a major role in increasing chlorophyll content thereby lead to increased photosynthetic accumulation. Chlorophyll recorded before harvest expresses on par in all treatments except nanoemulsion zeatin @ 5ppm and control.

Yield attributes (Table 2) of number of clusters is not significantly different among treatments, though numerical increase was observed compared to control in all other treatments. Effect of NAA in increasing number of clusters is recorded in mungbean [30]. A type of cytokinin, 6-BA reported increased number of cluster buds in *Houttuynia cordata* [31]. Immature pods present before harvest showed significant difference among treatments. Nanoemulsion zeatin @ 2 ppm (37.53) gave the highest immature pods followed by Conventional formulation of Zeatin @ 5ppm (31.90). Control recorded very less immature pods (8.49) which may be due to increased flower shedding leading to decreased pod setting. Number of pods (mature and immature pods), number of seeds, seed weight and number of flowers during harvest were significantly influenced by the application of plant growth regulators compared to control.

Highest mature pods is recorded in Nanoemulsion of NAA @ 30ppm (73.61) compared to control (31.76). Total number of pods formed in plant is highest in Nanoemulsion of NAA @ 30ppm (93.25) and on par unit with Conventional formulation of Zeatin @ 5ppm (90.77). This finding was similar to that of NAA spray at 3 split doses on 45, 90 and 130 DAS recorded 11.5% increase in pod yield in chickpea [32]. Increased mature pods by auxin which present in the shoot tips involve in the regulation of source-sink relationship by mediating the flow of metabolites [33]. The effect of cytokinin in increasing pod number in soybean was also proved which may be due to increased availability of assimilates to reproductive organ [34].

Spraying of Nanoemulsion of NAA 30@ ppm (20.68%) reduced flower shedding percentage followed by conventional formulation of Zeatin @ 5ppm. Highest flower shedding percentage was recoded in control (52.57%). Similar finding was proved in blackgram with NAA application @5, 10 and 15 ppm respectively [35]. Effect of NAA foliar spray in pigeon pea reported reduced flower drop per plant [36] and broad bean (Sharief and Hamady. 2017). Reduced flower abscission (31.17, 32.26 and 31.89 percent) due to cytokinin was also recorded in lentil due to foliar application of kinetin @ 10, 20 and 40 ppm compared to control with flower shedding percentage of 40.28 percent [37].

Pod setting percentage showed superiority in Nanoemulsion of NAA @ 2ppm (80.70%) followed by Nanoemulsion of Zeatin @ 2 ppm (73.63%). Similar result of 26 percent increased pod setting percentage was observed in soybean by foliar application of NAA @ 10 ppm [27]. This increase in pod setting mediated by NAA application may be due to reduced flower abscission which favoured formation of pods and effect of auxin in mediating flow of metabolites from source to sink may be the reason [33]. Benzyl Adenine applied to the racemes of soybean markedly increased the pod-set percentage in apical flowers of Essex and Shore varieties [38]. Flower shedding percentage and pod setting percentage has been presented in Fig. 1.

Total number of seeds per plant is higher in Nanoemulsion of NAA @ 30ppm (591.12) and it was comparative unit by Conventional formulation of Zeatin @ 5ppm (526.56). This might be due to increased number of pods in respective treatments. The research finding is correlated with the finding of increased number of pod and seed weight with the application of NAA @ 20ppm in soybean [39] and increased seed yield in blackgram through foliar spray of NAA @ 5, 10 and 15 ppm [35]. Effect of cytokinin in increasing seed yield due to increased sink strength has been reported in *Arabidopsis thaliana* [18]. Increased seed yield (3.83, 4.23 and 4.16) due to kinetin @ 0.01, 1 and 100 μM compared control (3.73 g/plant) in greengram [25].

Higher seed weight (17.95) was observed due to application of Nanoemulsion of NAA @ 30ppm followed by conventional formulation of Zeatin @ 5ppm which was on par with all other treatments except control. Increased number of seeds pod^{-1} and seed weight plant^{-1} by the application of NAA and zeatin were due to increased leaf area which attribute to the synthesis of photo assimilates at higher concentration, which lead to increase source. Moreover increased translocation of photo assimilates from source to sink stimulated by presence of auxin in sink region is also the reason for increase in number of seeds pod^{-1} and seed weight plant^{-1}. Similar results was observed due to spray of NAA @ 20 ppm recorded higher seed weight (14.27g) compared to control in soybean (13.85) due to increased pods plant^{-1} and seeds pod^{-1} [39]. An increase of yield due to increased growth and yield parameters in blackgram was reported due spray of Planofix (NAA) [28]. Also spray of kinetin @ 40 ppm and 10 ppm recorded increased seed weight in lentil [37]. The effect of benzyladenine in soybean reported increased seed weight per plant [40].

4. CONCLUSION

Nanoemulsion of NAA @ 30ppm recorded increased chlorophyll content which leads to increased no of clusters, number of pods/plant, no of seeds/plant, seed weight/plant, pod setting percentage with decreased flower shedding percentage. Hence, NAA nanoemulsion @ 30 ppm is best treatment among all other treatments in post flowering management to increase productivity of greengram.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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