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Effect of Integrating Plant Growth Regulators and Micronutrients on Growth, Phenology and Seed Yield of Fenugreek (*Trigonella foenum-graecum* L.)

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Authors' contributions

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

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ABSTRACT

An experiment was conducted during *Rabi* 2019-20 at College of Agriculture, Indore (Madhya Pradesh) to investigate the effects of Plant Growth Regulator (PGRs) and Micro-nutrient Application on Growth, Quality, and Seed yield of Fenugreek (*Trigonella foenum-graecum* L.). The nine

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treatments consisted of different levels of PGRs i.e. GA_3 and NAA with micro-nutrient (Mixol) which contain zinc, iron, manganese copper, boron and molybdenum including control (No use of PGRs and micro-nutrients) viz. T_1 = 50 ppm GA_3 seed treatment + Mixol 1.0%, T_2 = 100 ppm GA_3 seed treatment + Mixol 1.0%, T_3 = 50 ppm GA_3 foliar spray + Mixol 1.0%, T_4 = 100 ppm GA_3 foliar spray + Mixol 1.0%, T_5 = 25 ppm NAA seed treatment + Mixol 1.0%, T_6 = 50 ppm NAA seed treatment + Mixol 1.0%, T_7=25 ppm foliar spray + Mixol 1.0%, T_8 = 50 ppm NAA foliar spray + Mixol 1.0% and T_9 = Control. Data were collected on different growth characters (Plant height and number of branches), Phonological attributes (Days to first flowering, days taken to 50% flowering and days to taken seed maturity) and yield characters (Seed yield per plant (g), seed yield t/ha, number of pods per plant, length of pod (cm), number of seeds per pod) of fenugreek. Based on results of one year experimentation, foliar spray of GA_3 at 100 ppm + mixol 1.0% was found the best treatment compared with respect to plant growth and seed yield of fenugreek.

Keywords: Micro-nutrient (Mixol); PGRs; GA_3; NAA; foliar spray; Fenugreek.

1. INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an important vegetable grown in tropical and subtropical regions worldwide. It is an annual crop belonging to the Leguminosae (Fabaceae) family and the Faboideae subfamily. India is known as the "Land of Spices" and is a major producer, consumer, and exporter of spices. Fenugreek ranks third in world spice production after coriander and cumin. It is used as a herb (fresh leaves or dried) and spice (seeds) (Zandi et al. 2015). Out of 63 spices cultivated in India, 20 are classified as spice seeds, accounting for 36% of all spices cultivated and 17% of the country's spice production (Anwer et al., 2011). In India, area under fenugreek is 93,125 ha with production of 12,10,845 tonnes of seed (Anonymous, 2017-18). Rajasthan covered maximum area and production under fenugreek cultivation. It contributes 80 percent area and production of the country alone. Madhya Pradesh shared 10.12 percent in the total production of India (Anonymous, 2017). The major districts where fenugreek is cultivated include Jabalpur, Chhatarpur, Indore, Mandsaur, Nimach, Sehore and Sagar. Fenugreek is an important legume used as a vegetable, spice and herb, its fresh and dried leaves and seeds are consumed worldwide (Lewis et al., 2005). It is assumed to possess nutritive and restorative properties. The young leaves and sprouts are good source of protein, minerals, and vitamin-C (Khan et al., 2005 and Chhibba et al., 2007).

Plant growth regulators (PGRs) play critical roles in various physiological processes, where their specific concentrations are responsible for either promoting or inhibiting growth (Kumar et al., 2018). PGRs are known to significantly influence growth and development at high concentrations (Patel et al., 2018). They are essential for enhancing crop production and quality, and can be categorized into five major classes: auxins, gibberellins, cytokinins, abscisic acid, and ethylene. Gibberellic acid (GA_3), a key growth-stimulating substance, aids in increasing stalk length, enhancing vegetative growth, initiating flowering, improving fruit size, hastening maturity, and improving fruit quality in various crops, Concentrations of GA_3 50 ppm and 100 ppm are commonly used in agricultural research due to their effectiveness in enhancing plant height, root development, and overall vigor. (Swamy, 2012; Haq et al., 2013). Similarly, synthetic auxin, such as Naphthalene Acetic Acid (NAA), is important in processes like cell elongation, cell division, vascular tissue differentiation, root inhibition, apical dominance, leaf senescence, leaf and fruit abscission, fruit setting, and flowering. Typical concentrations of NAA in plant treatments range from 25 ppm to 100 ppm depending on the crop, with lower concentrations (such as 25 ppm and 50 ppm) being used for root stimulation and early growth phases (Singh et al., 2020). Micronutrients play a crucial role in enzyme activation and other physiological processes in plants. Micronutrient combinations like Mixol, which typically contain essential elements like zinc, iron, manganese copper, boron and molybdenum, help in enhancing photosynthesis, chlorophyll formation, and overall plant health (Alloway, 2008). The 1.0% concentration is a balanced and effective dose to supplement the PGR treatments and further boost the plant's growth and yield. Foliar application of PGRs has proven to be highly effective in increasing vegetative growth, early fruiting, total yield, and quality of fruits in many vegetable and spice crops (Krishnaveni et al., 2014). This study aims to explore the potential of integrating PGRs and micronutrients to improve the overall growth, phenology, and seed yield of fenugreek. By applying different concentrations of GA3 and NAA in combination with the micronutrient solution Mixol.

2. MATERIALS AND METHODS

The experiment was carried out at the Experimental Unit of the College of Agriculture, Indore Madhya Pradesh, during Rabi season, 2019-20. The study area is geographically situated at $22^{0}43'$ North latitude and $75^{0}66'$ East longitudes with an altitude of 556 meters above the sea level. The region falls under the agro-climatic Zone-I, known as the Malwa Plateau, which is part of Western Madhya Pradesh. The climate of this zone is characterized as sub-tropical, with moderately cold winters and unpredictable winter rains. During summer, temperatures range between 21°C and 44°C, while in winter, they vary from 6°C to 32°C. The rainfall occurs mostly from mid-June to the end of September. The South-West monsoon is responsible for major part of annual precipitation and annual mean rainfall is 965 mm. The soil in the experimental plot is classified as medium black clay (Vertisols), which is known for its relatively high fertility and ability to retain moisture. The surface of the soil is static, with good drainage capacity, making it suitable for fenugreek cultivation.

Table 1. Treatment combinations

Symbols	Treatments details
T_1	GA_3 at 50 ppm + seed treatment + Mixol 1.0%
T_2	GA_3 at 100 ppm + seed treatment + Mixol 1.0%
T_3	GA_3 at 50 ppm + foliar spray + Mixol 1.0%
T_4	GA_3 at 100 ppm + foliar spray + Mixol 1.0%
T_5	NAA at 25 ppm + seed treatment + Mixol 1.0%
T_6	NAA at 50 ppm + seed treatment + Mixol 1.0%
T_7	NAA at 25 ppm + foliar spray + Mixol 1.0%
T_8	NAA at 50 ppm + foliar spray + Mixol 1.0%
T_9	Control (No use of PGRs and micro-nutrients)

The experimental design followed a randomized block design (RBD) and consisted of three replications and nine treatments viz., GA3 (50 and 100 ppm), NAA (50 and 100 ppm) both are used as seed treatment and foliar spray with Mixol 1.0 % concentration and No use of PGRs and micro-nutrients as control. Spraying of plant growth regulators and micro-nutrient were done at 30 and 35 days after sowing.

3. RESULTS AND DISCUSSION

3.1 Growth Attribute

3.1.1 Plant height

The study evaluated the effects of two plant growth regulators (PGRs) and micronutrient treatments on fenugreek plant height at harvest. Significant variations in plant height were observed among treatments, as summarized in Table 2 and Fig. 1. Among the treatments, the highest plant height 106.41 cm was obtained with T4, which consisted of a foliar spray of GA_3 at 100 ppm with 1.0% Mixol, which showed significant increase in plant height to all other treatments. This result is consistent with earlier findings that GA_3, a gibberellin, promotes stem elongation and overall plant height by stimulating cell division and elongation (Chaudhary et al., 2015). The second highest plant height was recorded with T8, where NAA at 50 ppm with 1.0% Mixol was that recorded 106.16 cm. NAA, an auxin, is known to affect cell elongation and can synergistically react with micronutrients to enhance plant growth (Khan et al., 2017). In contrast, the lowest plant height was observed in the control treatment T9, which received only clean water without PGR or micronutrient application. This indicates that the absence of PGRs and micronutrients resulted in less vigorous growth, highlighting their importance in promoting plant height. These findings match previous research showing that plant growth regulators and micronutrients are important for optimal plant growth and can significantly enhance growth parameters (Ali et al., 2016). The observed increase in plant height with GA_3 application can be attributed to its role in promoting cell elongation and space. (Gholami et al. 2018, Sharma et al. 2006) reported that high concentrations of GA_3 positively affected plant height in various crops.

3.1.2 Branches per plant

The results showed significant variation in the number of branches per plant, which is affected by different concentrations of GA_3 and NAA in combination with micronutrients. Treatment T4, which consisted of a foliar spray of GA_3 at 100 ppm with 1.0% Mixol, resulted in the highest number of branches per plant. Specifically, T4 produced 24.50 branches at harvest time, as shown in Table 2 and Fig. 1. These results were significantly better than all other treatments. The increased branching observed in T4 can be attributed to the role of GA_3 in promoting cell division and elongation, which facilitates increased branch growth (Khan et al., 2018, Bakker et al., 2015). Treatment T8, which contained NAA at 50 ppm with 1.0% Mixol, also demonstrated an increase in the number of branches compared to most other treatments but was not as pronounced as T4. The observed increase in branching with NAA is consistent with its known effects on promoting lateral bud development and enhancing branch formation (Choudhary et al., 2016). In contrast, the control treatment T9 in which no PGRs or micronutrients were used showed the least number of branches per plant, with 19.73 branches at harvest. Similarly, T1 with GA3 at 50 ppm as a seed treatment combined with Mixol also displayed lower number of branches than T4, indicating that lower concentrations of GA_3 were less effective in promoting branch growth (Sharma et al., 2006, Sunanda et al. 2014, Ali et al., 2018).

3.2 Phonological Attributes

3.2.1 Number of days to 1st flowering

The results showed in Table 2 and Fig. 1 that there was no significant difference in the number of days taken to first flowering in different treatments, indicating that the application of GA_3 and NAA along with micronutrients did not cause any major change in the time taken to flowering. However, the highest number of days taken to first flowering was recorded in the T4 treatment, which involved foliar spray of GA_3 at 100 ppm with 1.0% mixol, with an average of 51.22 days. In contrast, the lowest number of days taken to first flowering was observed in the control treatment, T9, which did not receive any PGR or micronutrients, with an average of 46.44 days. These findings suggest a slight delay in flowering due to GA_3 treatment, although the difference was not statistically significant. The results are consistent with previous research showing that gibberellins such as GA_3 can sometimes delay flowering in certain plant species due to their role in extending the vegetative phase and promoting growth (Al-Ghamdi et al., 2017). This delay can be attributed to the effect of GA_3 on promoting vegetative growth and increasing the time required for plants to transition from the vegetative to the reproductive phase (Khan et al., 2016).

3.2.2 Number of days to 50% flowering

The results as illustrated in Table 2, showed that the application of different levels of GA_3 and NAA along with micronutrients did not cause any significant difference in days to 50% flowering. This indicates that while these treatments may affect other growth parameters, their effect on the time to 50% flowering was minimal. Despite the lack of significant variation, notable trends were observed. Treatment T4, which involved foliar spray of GA_3 at 100 ppm with 1.0% Mixol, resulted in the highest number of days to 50% flowering, averaging 60.09 days, This trend highlighted in Fig. 1. This finding suggests that high concentrations of GA_3 may cause a delay in flowering. The delay in flowering associated with GA_3 application may be attributed to its role in extending the vegetative growth phase and promoting overall plant growth, which may subsequently postpone the reproductive phase (Ali et al., 2018). Similar results have been reported in previous studies, where high levels of gibberellins were found to increase flowering time due to increased vegetative growth (Moein et al., 2016). In contrast, the least number of days to 50% flowering was observed in the control treatment, T9, in which no PGRs or micronutrients were used, averaging 55.63 days.

3.2.3 Number of days taken to seed maturity

The findings as illustrated in Table 2, showed that the treatments did not result in any significant difference in the number of days required for seed maturity. This suggests that the application of GA_3 and NAA along with micronutrients had minimal effect on the duration of the reproductive stage in fenugreek. Despite the lack of statistical significance, treatment T4, which involved foliar spray of GA_3 at 100 ppm with 1.0% Mixol, resulted in the highest number of days taken for seed maturity, averaging 118.15 days. This trend highlighted in Fig. 1, indicates that higher concentrations of GA_3 may slightly increase the time to seed maturity. Gibberellins, like GA_3, are also known to promote vegetative growth, which may result in a longer growth cycle before fully transitioning to the reproductive stage (Choudhary et al., 2017). This delay in seed maturity may be due to the ability of GA_3 to stimulate vegetative vigour, thereby increasing the time taken for the plant to complete its life cycle and reach seed maturity (Moein et al., 2016) and the shortest number of days to seed maturity was observed in the control treatment, T9, which did not involve the use of PGRs or micronutrients. The control plants matured in an average of 111.17 days, indicating that the absence of PGRs and micronutrients led to a shorter time for seed maturity.

3.3 Yield Characteristics

3.3.1 Seed yield per plant

The application of different levels of plant growth regulators (PGRs), including GA_3 and NAA in combination with Mixol (1.0% micronutrient solution), had a significant effect on seed yield per plant. According to the data presented in Table 3, the highest seed yield per plant (13.92 g) was observed in treatment T4, which consisted of foliar spray of 100 ppm GA_3 with Mixol (1.0%). This result is further visualized in Fig. 2, where the seed yield data across all treatments are illustrated. Treatment T4 was closely followed by treatment T8 (50 ppm NAA foliar spray with Mixol), indicating the beneficial effects of these PGRs on improving seed yield. The increase in seed yield observed in T4 treatment can be attributed to the role of GA_3 in enhancing plant growth and development. Gibberellins, such as GA_3, are known to stimulate various physiological processes, including cell elongation, enzyme activation, and nutrient mobilization, all of which contribute to enhanced reproductive growth and seed set (Khan et al., 2016. Ali et al., 2015). Treatment T8, which consisted of 50 ppm foliar spray of NAA combined with Mixol, resulted in a seed yield of 12.86 g per plant. This relatively high seed yield suggests that NAA, an auxin, also plays an important role in enhancing reproductive growth by promoting cell division and fruit/seed development (Gholami et al., 2017).). In contrast, the lowest seed yield (9.56 g) per plant was recorded in the control treatment (T9) which did not receive any PGRs or micronutrients. Similar results were found in Mehta et al. (2012) and Choudhary et al., (2014) in fenugreek.

3.3.2 Seed yield quintal per hectare

The highest seed yield per hectare (1.524 tonnes/ha) was observed in treatment T4, which involved foliar spray of 100 ppm GA_3 with Mixol 1.0%, as shown in Table 3. This was closely followed by treatment T8, which involved foliar spray of 50 ppm NAA with Mixol, yielding 1.508 tonnes/ha. The control treatment, T9, which did not add any PGR or micronutrients, produced the lowest seed yield per hectare (1.232 tonnes/ha). The significant increase in seed yield under treatment T4 can be attributed to the positive effects of GA_3 on both vegetative and reproductive growth, which is further illustrated in Fig. 2, where the comparative seed yield of different treatments is depicted. GA_3 promotes cell growth, enhances nutrient translocation, and improves overall plant vigour, which collectively contributes to higher seed yield (Choudhary et al., 2016). The result of 1.524 tonnes/ha from T4 is consistent with previous research showing that foliar application of GA_3 increases seed yield in legumes and other crops (Mehta et al. 2012. Khan et al., 2018. Moein et al., 2017). Treatment T8 (50 ppm NAA with Mixol) resulted in significantly higher seed yield (1.508 tonnes/ha), indicating that NAA, an auxin, plays an important role in promoting reproductive growth and seed yield in fenugreek. NAA stimulates root growth, enhances nutrient absorption, and supports fruit and seed development, which contribute to substantial increases in yield (Ali et al., 2015). The control treatment, T9, which did not receive any PGRs or micronutrients, resulted in the lowest seed yield per hectare (1.232 tonnes/ha). This finding emphasizes the importance of exogenous application of PGRs and micronutrients to optimize seed yield. Similar results were found in Mehta et al. (2012) in fenugreek, Sahu et al. (2012), and Hnamte et al. (2013) in coriander.

3.3.3 Number of pods per plant

The results showed that the highest number of pods per plant (86.18) was recorded from treatment T4, which consisted of foliar spray of 100 ppm GA_3 with Mixol (1.0%), as displayed in Table 3. This was significantly higher than all other treatments and was followed by treatment T8 (50 ppm NAA foliar spray with Mixol), which recorded 84.76 pods per plant. The significant increase in the number of pods per plant in treatment T4 can be attributed to the positive effect of GA_3 on enhancing both vegetative and reproductive growth, a result further illustrated in Fig. 2. Treatment T8, which involved 50 ppm NAA foliar spray, resulted in a slightly lower number of pods per plant (84.76) than T4. NAA, an auxin, is known to promote cell division and pod formation by maintaining hormonal balance during reproductive growth stages (Chaudhary et al., 2014). The relatively high number of pods in

T8 indicates the importance of auxins in supporting reproductive development, although the results suggest that GA_3 at 100 ppm is more effective in increasing the number of pods. In contrast, the lowest number of pods per plant (81.27) was found in the control treatment (T9), which did not receive any PGR or micronutrients. The control treatment was followed by T1 (50 ppm GA_3 seed treatment with Mixol), T5 (25 ppm NAA seed treatment with Mixol), and T6 (50 ppm NAA seed treatment with Mixol), all of which recorded lower pod numbers than the foliar treatments. Similar results for most of the characters were also reported by Bairva et al. (2012), Gendy et al. (2013), Naimuddin et al. (2014), Krishnaveni et al. (2016), Godara et al. (2018).

3.3.4 Length of pod (cm)

The results indicated that the highest pod length (15.27 cm) was observed from treatment T4, which consisted of foliar spray of 100 ppm GA_3 with Mixol 1.0%, as presented in Table 3. This result was significantly different from all other treatments but was quite close to treatments T8 (50 ppm NAA foliar spray with Mixol) and T2 (100 ppm GA3 seed treatment with Mixol 1.0%), which showed pod lengths of 14.95 cm and 14.57 cm, respectively. The significant increase in pod length under treatment T4 can be attributed to the beneficial effects of GA_3 in promoting cell growth and overall vegetative growth, including the development of reproductive organs such as pods (Khan et al., 2016), as shown in Fig. 2. Foliar application of GA_3 at 100 ppm possibly provides optimum hormonal conditions for pod growth, ensuring better pod development and yield potential. Treatment T8 (50 ppm NAA foliar spray with Mixol) also resulted in a significant increase in pod length (14.95 cm), suggesting that auxins such as NAA help to maintain proper hormonal balance, which is important for optimal pod growth. The results are consistent with previous findings showing that auxins, especially when applied as foliar sprays, effectively improve pod length and other yield components in legume crops (Chaudhary et al., 2016). The control treatment T9, which did not receive any PGR or micronutrients, displayed the lowest pod length (10.43 cm). This was statistically similar to the pod length recorded in treatments T1 (50 ppm GA3 seed treatment with Mixol 1.0%), T5 (25 ppm NAA seed treatment with Mixol 1.0%), and T6 (50 ppm NAA seed treatment with Mixol 1.0%). Similar results have been reported in previous research, where lack of hormonal stimulation led to sub-optimal pod development in beans (Gholami et al., 2017).

3.3.5 Number of seeds per pod

The highest number of seeds per pod (16.54) was recorded from treatment T4, which consisted of foliar spray of 100 ppm GA_3 with Mixol 1.0%, as presented in Table 3 and Fig. 2. This result was statistically similar to T8 (50 ppm NAA foliar spray with Mixol 1.0%) and statistically similar to T3 (50 ppm GA_3 foliar spray with Mixol 1.0%) and T7 (25 ppm NAA foliar spray with Mixol 1.0%). The increased number of seeds per pod in treatment T4 suggests that the application of GA_3 at 100 ppm significantly enhances reproductive growth, possibly by improving flower and pod set and by enhancing seed development (Khan et al., 2016, Ali et al., 2015). Similarly, treatment T8 (50 ppm NAA foliar spray) also recorded a higher number of seeds per pod, indicating that auxins like NAA play a vital role in enhancing seed formation by promoting proper cell division and pod growth (Choudhary et al., 2016, Sharanya et al. (2018) and Reddy et al. 2020). Control treatment T9, which did not receive any plant growth regulator (PGR) or micronutrient, recorded the lowest number of seeds per pod (11.43). The control was followed by T1 (50 ppm GA_3 foliar spray with Mixol 1.0%), which showed a relatively lower seed number compared to higher PGR concentrations (Moein et al., 2018).

Table 2. Effect of different treatments on growth and phenological attributes of Fenugreek

Sym	Plant height (cm)	Number of branches	Number of days of first flowering	Number of days to 50% flowering	Days taken to maturity
T_1	98.63	20.57	47.65	56.29	113.10
T_2	104.42	21.10	48.07	58.87	114.12
T_3	101.77	22.83	49.84	59.64	117.43
T_4	106.41	24.50	51.22	60.09	118.15
T_5	99.82	20.50	47.13	57.34	113.59
T_6	100.80	21.10	47.89	58.22	115.25

Sym	Plant height (cm)	Number of branches	Number of days of first flowering	Number of days to 50% flowering	Days taken to maturity
T_7	102.43	21.20	49.35	59.11	116.92
T_8	106.16	23.73	50.69	59.92	117.99
T_9	96.16	19.73	46.44	55.63	111.17
SEm±	**1.72**	**1.72**	**1.59**	**1.94**	**1.69**
CD(P=0.05)	**5.10**	**5.12**	**4.74**	**5.77**	**5.01**

Table 3. Effect of different treatments on yield attributes of Fenugreek

Sym.	Seed yield(g/plant)	Seed yield(q/ha)	No. of pods/plant	Length of pod (cm)	No. of seeds/pod
T_1	9.76	1.256	82.73	11.40	12.67
T_2	11.82	1.412	84.67	14.27	14.07
T_3	12.67	1.488	85.46	12.87	15.47
T_4	13.92	1.524	86.18	15.27	16.81
T_5	10.49	1.342	83.07	11.80	12.20
T_6	11.26	1.384	84.42	12.27	12.93
T_7	12.26	1.444	85.16	13.07	14.77
T_8	13.13	1.508	85.93	15.00	16.43
T_9	9.56	1.232	81.27	10.43	11.43
SEm±	**1.01**	**0.70**	**2.45**	**1.11**	**1.31**
CD(P=0.05)	**2.99**	**2.08**	**7.27**	**3.31**	**3.88**

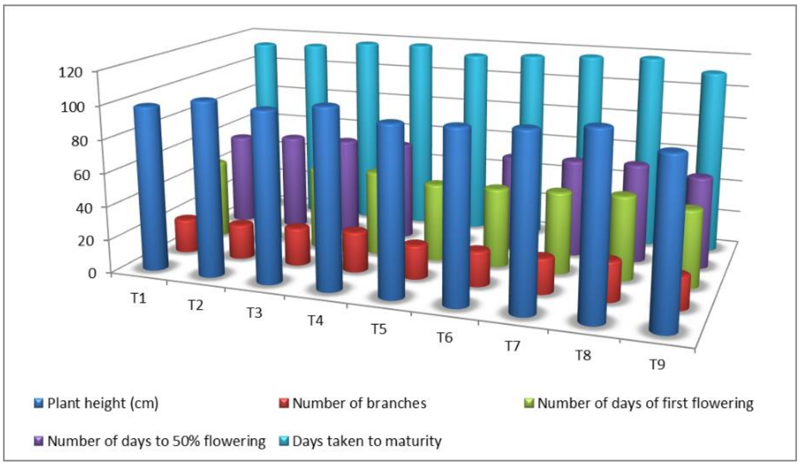

Fig. 1. Effect of different treatments on growth and phenological attributes of Fenugreek

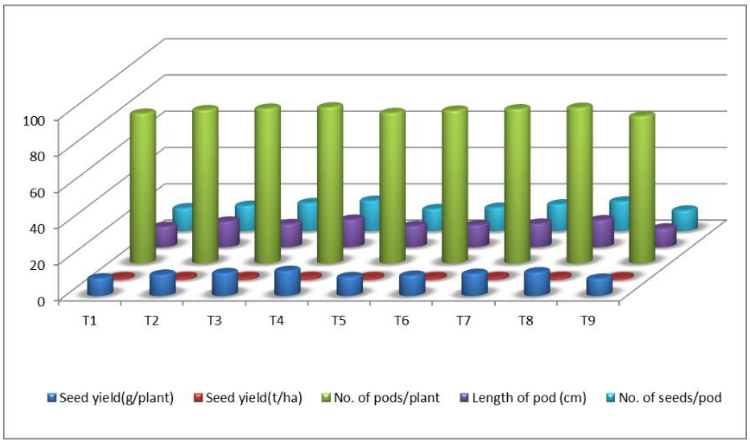

Fig. 2. Impact of different treatments on yield characteristics of Fenugreek

4. CONCLUSION

Current study has shown that plant growth regulators (such as GA_3 and NAA) used in combination with micronutrients (Zn, Fe, Mn, Cu, B, Mo) can affect many growth, phenological and yield traits of fenugreek. T4 treatment, which included foliar sprays of 100 ppm GA_3 and 1.0% Mixol, consistently outperformed the other treatments, resulting in the highest plant height, branch count, pod length, seed yield per plant and total biomass yield. This suggests that GA_3 plays a role in promoting cell division and elongation, helping to improve growth and development. NAA at 50 ppm in combination with Mixol (T8) also showed a positive effect on growth retardation, but to a lesser extent than T4. In contrast, the control treatment (T9) without plant growth regulators and micronutrients showed the lowest values of most parameters, referring to the importance of inputs for visual growth and yield. Although the effects of the treatments on flowering time and seed maturation were small, their significant effects on good results such as seed yield and harvest indicate that the use of eight of the plant growth regulators and micronutrients can increase fenugreek productivity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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