Enhancement of growth, yield and quality of *Trigonella foenum-graecum* L. in response to dual application of *Azotobacter chrococcum* and *Pseudomonas fluorescens* in Northern India

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Abstract. Medicinal plants are used in natural therapies and food additives, but chemical fertilizers can negatively impact plant growth. Using beneficial rhizosphere microbiota can improve plant production under both conventional and stressed conditions. The present investigation was was set up using a Randomized Block Design (RBD), with six treatments and three replications each, carried out during the rabi season of 2024. Treatments as T1 (Control), T2 (Vermicompost at 5 t/ha), T3 (Azotobacter at 15 g/kg seed + Vermicompost at 2.5 t / ha), T4 (PSB + Azotobacter at 15 g / kg seed), T5 (FYM at 10 t / ha), and T6 (FYM at 5 t / ha + Azotobacter + PSB at 15 g / kg seed) investigated the impact of bio-fertilizers on plant biometric parameters and crude protein content in fenugreek. The plant's total performance was greatly enhanced by applying both Azotobacter and PSB in addition to FYM. The results showed that the treatment T6 (FYM at 5 t / ha + Azotobacter + PSB at 15 g / kg seed) significantly increased plant height, number of branches, number of leaves and yield compared to the control at all time points (30 DAS, 60 DAS, and 90 DAS), followed by treatment T4 (PSB + Azotobacter at 15 g/kg seed). The treatment T6 yields the highest crude protein content, up to 21.94%, followed by the treatment T4 as a reasonably high protein content of 21.32%. The study revealed that Azotobacter and PSB, along with FYM, significantly improved plant performance, suggesting that beneficial microorganisms can enhance plant growth and development. Fertilizers such as FYM and vermicompost also had a positive impact on plant growth and quality in fenugreek.

Keywords: Biofertilizer; crude protein; organic farming; *Trigonella foenum-graecum* L.; yield.

1. Introduction

Fenugreek, also known as *Trigonella foenum-graecum* L., is a diploid plant in the Fabaceae family, the oldest known medicinal plant. It is grown globally, including India, North Africa, and the Middle East, and is used in traditional medicine for its medicinal properties [1]. Fenugreek is also used in food, cosmetics, and pharmaceuticals [2]. Its leaves and seeds are used for flavoring and spices, and its seeds can enhance food's flavor and strengthen the immune system [3]. Fenugreek also has pharmacological qualities, including antibacterial, carminative, emollient, febrifuge, laxative, restorative, galactogogue, expectoral, antiviral, antioxidant, demulcent, and hypotensive effects [4].

Owing to intensive farming practices aimed at boosting output and efficiency, a significant amount of chemical fertilizers are being used, which is causing environmental contamination and soil degradation. When compared to the amount of nutrients that the plants were removing, the fertilizer application typically stayed far lower. The amount of nutrients given and extracted from the soil differs greatly as a result. The use of inexpensive organic additions like biofertilizer in conjunction with chemical fertilizers could close this difference.

Chemical fertilizers alone may not always be as beneficial as using biofertilizers in conjunction with fenugreek. Biofertilizer management in fenugreek can help to reduce the amount of chemical fertilizer required. The usage of chemical fertilizers has decreased as a result of the solicitation of organic manure with biofertilizers, which has also produced high-quality products free of harmful agrochemicals [5, 6]. Rhizobium and phosphate solubilizing bacteria (PSB) in conjunction with microbial fertilizer have been demonstrated to improve crop productivity and soil health.

Using biofertilizers promotes healthy plant growth and improves soil health. Organic nutrients that meet plant nutrient requirements and improve soil fertility are provided by microorganisms and their metabolites. Studies have demonstrated that introducing Rhizobium into seeds can enhance their development, yield, and quality in fenugreek and green gram [7].
Microbial fertilizers are significant agricultural inputs because they are environmentally friendly, low in cost, and non-bulky. Reliance on chemical fertilizers can be decreased by using bacterial fertilizers as a source of N and P. While Rhizobium can fix atmospheric nitrogen, PSB can solubilize and mobilize P and other inaccessible micronutrients found in the soil [8]. In addition, biofertilizers are becoming more and more popular as a sustainable and environmentally friendly means of enhancing plant growth and nutrient availability. Biofertilizers have demonstrated promise in boosting soil fertility and plant nutrient uptake because they contain helpful microbes like nitrogen-fixing bacteria. Biofertilizer increases soil efficiency by either fixing nitrogen from the atmosphere or stimulating plant development by synthesizing chemicals that aid in growth [9]. Thus, it is imperative to use organic and inorganic sources with caution and appropriateness in order to maintain soil health and sustainability for a long time, as well as to increase profits and produce of higher quality [10]. Therefore, it is especially important to use organic resources like green manure, FYM, and biofertilizer as they lower production costs, maintain soil health, and are easily available to small and marginal growers.

The current research was carried out with the following aims and objectives:

- To study the application of biofertilizers and their impact on the growth and yield of fenugreek.
- To analyse the quality (crude protein) of fenugreek following various treatments.

2. Material and methods

2.1 Experimental details

The study aimed to improve the growth, yield, and quality of *Trigonella foenum-graecum* L. by utilizing *Azotobacter chrococcum* and *Pseudomonas fluorescens* in the Northern region, India during rabi season 2024. This experiment investigated the impacts of using azotobacter and phosphate-solubilizing bacteria at the same time, as well as how these effects affected the overall performance of *Trigonella foenum graecum* L. The experiment was set up using a randomized block design with several treatments. Treatment details is provided in Table 1.

Table 1: Treatment details and their dose application on the field trials

Treatment	Treatment
T1	Control treatment
T2	Vermicompost (5 t / ha)
T3	Azotobacter at 15g/kg seed + Vermicompost at 2.5 t/ha
T4	PSB + Azotobacter at 15 g/kg seed
T5	FYM 10 t/ha
T6	FYM at 5 t/ha + Azotobacter + PSB 15 g/kg seed

2.2 Experimental layout

Trigonella foenum-graecum L. (Fenugreek), a member of the Fabaceae family, was grown in the rabi season of 2024 using a Randomized Block Design (RBD) at the Amity Institute of Organic Agriculture Farm, Amity University in Noida. The overall area was 42 square meter, with area of plot measuring 7 sq. m and each plot measuring 3.5 m x 2 meter. Six treatments were used, each with three replications and 18 plots in total.

2.3 Observation recorded

To gain a better knowledge of the mechanics of plant growth and development, it was required to observe how the developing plant responded to various treatments. Five healthy plants were picked at random and each was given a tag to aid identification. The chosen plants were observed and noted at 30 DAS, 60 DAS and at 90 DAS respectively. The number of plants, branches, plant height, and yield were measured to see how different treatments affected plant growth. Design analysis of variance was performed using the conventional method recommended by Dutta et al. [8]. Analysing the effects of various treatments on a variety of plant properties, observations were made throughout the study:

2.3.1 Plant height

Three measurements of plant height were taken at 30 DAS, 60 DAS, and 90 DAS, considering the distance between soil surface and plant tips. The mean value for each treatment was determined. The number of branches was counted in each plot.

2.3.2 Number of leaves

The plants in each plot had their leaves manually counted during the crop's 30, 60 and at 90 DAS. The mean was calculated by taking the average of the values.

2.3.3 Leaf area

To get the leaf's overall surface area, the leaf length and breadth values were multiplied by taking the mean value for each treatment.

2.3.4 Plant weight

The weight of each plant is manually computed and weighed independently using a weighing machine. The plant weights were documented, and each plot's average weight was computed and recorded.

2.3.5 Number of pods

The study counted pods on tagged plants in each treatment, calculated the average pod number per plant, and randomly selected 5 pods from each plot to determine the average seed number per pod.

2.3.6 Pod length (cm)

Each plot's pod length was determined by averaging five randomly selected pods.

2.3.7 Pod yield (kg)

During harvest, the pod yields from each plot were weighed.

2.3.8 Crop yield (q/ha)

Crop yield per ha was recorded after the crop harvesting and recorded in quintal/hectare.

2.3.9 Quality (%)

The crude protein content was determined using a modified Kjeldahl method. The seeds were hydrolyzed with 20 ml of H_2SO_4 and a 3.5 g selenium catalyst tablet at 240°C for 30 minutes and 380°C for 3 hours. The samples were cooled. Prior to titration and neutralization, the hydrolysates were mixed with the required amounts of H_2O and NaOH. Proper laboratory techniques were followed to ensure accuracy and precision to calculate the crude protein content of the plant.

3. Results and discussion

3.1. Impact on growth parameters

Plant height is a crucial indicator of agricultural productivity. At 30 DAS, plant height varied from 11.83 cm to 13.17 cm. T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed) proved to be the most successful treatment, increasing plant height by 13.67 cm (Table 2). The treatment T3 (Azotobacter at 15 g/kg seed + Vermicompost at 2.5 t/ha) was statistically equivalent to T5 (FYM at 10 t/ha). The improvement in plant performance was attributed to the beneficial effects of Azotobacter and Pseudomonas, which naturally fix nitrogen and solubilize phosphate in the soil.

Table 2: Effect of different treatments on plant height in fenugreek

Tr. No.	Treatments	30 DAS	60 DAS	90 DAS
T1	Control treatment	13	22	25.40
T2	Vermicompost (5 t/ha)	12.67	24.83	26.50
T3	(Azotobacter at 15g/kg seed + Vermicompost at 2.5 t/ha)	11.83	28.10	32.33
T4	(PSB + Azotobacter at 15 g/kg seed)	13.17	26.93	33.47
T5	(FYM 10 t/ha)	12.70	25.00	28.23
T6	(FYM at 5 t/ha + Azotobacter + PSB 15 g/kg seed)	13.67	29.50	34.27
	S Em ±	0.25	1.09	1.55
	CD at 5%	0.53	1.13	1.45

The study found that the treatment T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed) significantly outperformed the control treatment at all time points

(30 DAS, 60 DAS, and 90 DAS). The plant height increased proportionally from 30 DAS to 90 DAS (Table 2), with the rate of height gain accelerating initially before slowing down. The beneficial effects of Azotobacter and Pseudomonas, which fix nitrogen and solubilize phosphate in the soil, were observed to improve plant performance.

The number of branches on each plant was counted at regular intervals. The results showed that applying organic manures and biofertilizer enhanced the number of branches per plant (Table 3). The results revealed a significant variance in the number of branches per plant across all treatments. The largest number of branches per plant (8.63) was produced by treatment T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/seed), while the lowest number per plant (7.07) was produced by treatment T1 (Control).

Table 3: Impact of various treatments on the number of branches per plant in fenugreek

Tr. No.	Treatments	30 DAS	60 DAS	90 DAS
T1	Control treatment	2.40	4.67	7.07
T2	Vermicompost (5 t/ha)	2.17	4.33	7.27
T3	(Azotobacter at 15g/kg seed + Vermicompost at 2.5 t/ha)	2.70	4	7.17
T4	(PSB + Azotobacter at 15 g/kg seed)	3.57	5.33	8
T5	(FYM 10 t/ha)	2.97	3.67	7.63
T6	FYM at 5 t/ha + Azotobacter + PSB 15 g/kg seed	3.63	5.67	8.63
	S Em ±	0.24	0.31	0.24
	CD at 5%	0.41	0.22	0.60

At 90 DAS, the number of branches per plant varied significantly, with each showing an increase. T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed) and T4 (PSB + Azotobacter at 15 g/kg seed) had the most branches per plant, with (8.63) and (8), respectively, followed by T5 (FYM 10 t/ha) with 7.63. While the lowest was recorded at T1(control) with (7.07) number of branches followed by T2 (vermicompost at 5 t/ha) with (7.27) number of branches. [11-14] showed a similar pattern of increased branch number due to the concurrent treatment of Rhizobium and PSB.

The number of leaves per plant varied significantly between treatments. T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed) had the most leaves (14.67) after 30 days of growth, which was much more than T1 (Control), which had the fewest leaves (10.20) (Table 4). At 60 DAS, noticeable variations were observed in the number of leaves per plant for each treatment. Treatment T6 (FYM at 5 t/ha + Azotobacter + PSB at 15g/kg seed) had the highest number of leaves (37.63) while T5 (FYM at 10 t/ha) had the lowest number of leaves (27.63) followed by T1 (Control) with (27.83) number of leaves. Similarly, after 60 DAS, there were noticeable differences in the grade of leaves per plant among treatments. The treatments with the most leaves, T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed) and T4 (PSB + Azotobacter at 15 g/kg seed) with 49.39 and 48.8 leaves, while T1 (Control) had 42.07 leaves and T2 (Vermicompost at 5 t/ha) had 42.23 leaves, the least amount of leaves compared to the other treatments (Table 4).

Table 4: Impact of various treatments on the number of leaves per plant in fenugreek

Tr. No.	Treatments	30 DAS	60DAS	90 DAS
T1	Control treatment	10.20	27.83	42.07
T2	Vermicompost (5 t/ha)	11.03	27.97	42.23
T3	Azotobacter at 15g/kg seed + Vermicompost at 2.5 t/ha	11.87	29.50	45.07
T4	PSB + Azotobacter at 15 g/kg seed	13.97	35.20	48.8
T5	T5 –FYM 10 t/ha	11.13	27.63	47.03
T6	FYM at 5 t/ha + Azotobacter + PSB 15 g/kg seed	14.67	37.63	49.39
	S Em ±	0.72	1.77	1.29
	CD at 5%	1.39	1.09	0.87

Significant alterations were seen at 30, 60, and 90 DAS for every treatment (Table 5). T1 (Control) had the shortest leaf area of 55.73 cm2, while T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed) had the most leaf area of 69 cm2 among all treatments. The other treatments, T2 (vermicompost at 5 t/ha), T3 (azotobacter at 15 g/kg seed + vermicompost at 2.5 t/ha), T4 (PSB + Azotobacter at 15 g/kg seed), and T5 (FYM at 10 t/ha), significantly impacted the T1 (Control) treatment.

Table 5: Effects of various treatments on leaf area in fenugreek (in cm^2)

Tr. No.	Treatments	30 DAS	60 DAS	90 DAS
T1	Control treatment	29.47	45	55.73
T2	Vermicompost (5 t/ha)	29.57	44	56.07
T3	Azotobacter at 15g/kg seed + Vermicompost at 2.5 t/ha	32.97	46.53	58.60
T4	PSB + Azotobacter at 15 g/kg seed	35.57	48.53	60
T5	FYM 10 t/ha	32.27	45.77	58
T6	FYM at 5 t/ha + Azotobacter + PSB 15 g/kg seed	37	49.27	60
	S Em ±	1.25	0.83	0.75
	CD at 5%	0.93	0.64	0.57

3.2 Impact on yield attributing parameters

Different organic manures and biofertilizers significantly enhanced the production attributes of fenugreek. The statistical results (Table 6) showed that treatment T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed) outperformed treatment T1 (Control) in terms of pod number, pod length, and number of seeds. With the use of biofertilizers, the accessibility of nutrient levels for the plant to assimilate improved, which has a significant effect on yield attributing parameters. This can be the result of applying biofertilizers and organic manures together. They accelerate growth due to increased uptake of water and minerals like phosphorus and nitrogen, increased biological N fixation, increased synthesis of plant growth hormones, and increased availability of P, all of which improve biomass, yield, and yield characteristics [15]. The highest pod length was recorded in T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed) with 17.90 number of pods per plant. The increase in number of pods per plant is consistent with the findings of Saxenna and Singh [14] and Somdutt [16].

The effect of pod length was considerable, as seen by T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed) with a pod length of 12.70 cm, which was statistically equivalent to T4 (PSB + Azotobacter at 15 g/kg seed). T1 (Control) had the shortest pod length, measuring 9.97 cm. T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed) had the most seeds per pod (17.30), while T1 (Control) had the fewest (12.07), which was statistically similar to T2.

Table 6: Variations in the number of pods, number of seeds per pod, length of pod (cm), and yield of pods (q/ha) as a result of different treatments in fenugreek.

Tr. No.	Treatment	No. of pods per plant	No. of seeds per pod	Pod length (cm)	Pod yield (q/ha)
T1	Control treatment	10.43	12.07	9.97	17.37
T2	Vermicompost (5 t/ha)	12.03	12.97	10.20	18.06
T3	Azotobacter at 15g/kg seed + Vermicompost at 2.5 t/ha	14.03	13.70	11.00	18.69
T4	PSB + Azotobacter at 15 g/kg seed	16.07	15.20	12.53	19.50
T5	FYM 10 t/ha	13	13.07	11.20	17.74
T6	FYM at 5 t/ha + Azotobacter + PSB 15 g/kg seed	17.90	17.30	12.70	19.82
	S Em ±	1.11	0.77	0.46	0.40
	CD at 5 %	1.64	0.67	0.75	0.93

T6 (FYM 5 t/ha + Azotobacter + PSB at 15 g/kg seed) had the same number of pods per plant as T4 (PSB + Azotobacter at 15 g/kg seed), while T2 (Vermicompost at 5 t/ha), T3 (azotobacter at 15 g/kg seed + vermicompost at 2.5 t/ha), and T5 (FYM at 10 t/ha) were statistically equivalent. The number of seeds per pod demonstrated how bio-fertilizers work together to promote more seed production on pods, which results in improved yield of the plant (Table 6).

The highest yield, 19.82 q/ha, was obtained in T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed). T4 (PSB + Azotobacter at 15 g/kg seed) followed with 19.50 q/ha in pod yield. Chaudhary's (1999) results on fenugreek were comparable to these findings [17]. The lowest pod yield (17.37 q/ha) was recorded in T1(Control) and T2(Vermicompost at 5 t/ha) with yield of (18.06 q/ha). Several studies have found similar results for the majority of yield characteristics[18-20]. With the use of biofertilizers, the accessibility of nutrient levels for the plant to assimilate is improved, which has a significant effect on yield characteristics and results in maximal pod weight. The study reveals that the use of bio-fertilizer progressively improved the yield characteristics of legume crops. The results showed that pod weight/plant was improved dramatically.

3.3 Impact on quality of Fenugreek

Table 7 shows the crude protein concentration (%) of various treatments.

Table 7: Protein content in Fenugreek under different treatment

Tr. No.	Treatment	Crude protein (%)
T1	Control treatment	18.59
T2	Vermicompost (5 t/ha)	19.07
T3	Azotobacter at 15g/kg seed + Vermicompost at 2.5 t/ha	20.09
T4	PSB + Azotobacter at 15 g/kg seed	21.32
T5	FYM 10 t/ha	19.39
T6	FYM at 5 t/ha + Azotobacter + PSB 15 g/kg seed	21.94
	S Em ±	0.53
	CD at 5 %	0.48

Comparing the treatments, it is clear that T6 (FYM at 5 t/ha + Azotobacter + PSB at 15 g/kg seed) consistently produces the greatest crude protein content, with a value of 21.94 %. T4 (PSB + Azotobacter at 15 g/kg seed) has a reasonably high protein content of 21.32 % (Table 7). The control group (T1) has the lowest protein content with value of 18.59 %, whereas other treatments T2 (Vermicompost at 5 t/ha), T3 (azotobacter at 15 g/kg seed + vermicompost at 2.5 t/ha), and T5 (FYM at 10 t/ha) were statistically at par to each other. Several studies have shown similar results for the crude protein contents in fenugreek [21-24]. These findings suggest that application of biofertilizer and organic amendments can positively influence plant crude protein concentration, resulting in increased nutritional quality of the plant.

4. Discussion

Several research have looked at how biofertilizers affect Fenugreek growth, yield, and quality under various situations. The impact of various natural composts and biofertilizers on fenugreek growth and production. Vermicompost, Azotobacter, and phosphorus-solubilizing microorganisms were used together, and this combination significantly improved plant top, leaf matter, shoot and root period, sparkling and dry weight, and yield when compared to the manipulation and other remedies [25]. In fenugreek, combined Rhizobium and PSB inoculation of seed produced noticeably greater yield characteristics, yields, gross return, net return, and BCR compared to their individual application and control. By providing organic resources through symbiotic nitrogen fixation and changing insoluble phosphorus into soluble form, biofertilizers containing rhizobium and PSB increase soil fertility and plant nutritional requirements. This results in greater plant growth and development [4, 20, 26-29].

Increased nutrient uptake and greater resistance to drought and moisture stress are the results of biofertilizers, which also produce antibiotics and fix nitrogen, phosphate, and potassium solubilization or mineralization, release plant growth regulators, and decompose organic matter in the soil [30]. Biofertilizers promote plant development by fixing nitrogen, suppressing pathogens, and generating growth-regulating hormones [31]. Biological biofertilizers reduce the need for mineral fertilizers, provide high-quality products without harmful agrochemicals, improve soil health, and provide plants with a sustainable source of nutrients [32, 33]. The application of biofertilizers encourages the plant's vegetative growth, which is reflected in an increase in grain yield. These fertilizers have the capacity to fix nitrogen as well as release specific phytohormones that may encourage plant growth, nutrient absorption, and photosynthesis. All of the aforementioned plant processes are what increase grain yield, as previously observed by Garwal [34].

The use of biofertilizers significantly increased fenugreek's generative and vegetative development, as well as its ability to absorb nutrients. Therefore, it is advisable to fertilize fenugreek plants with biofertilizer. Biofertilizers improved root structure and increased nutrient absorption. Another study demonstrated that biofertilizers greatly improve fenugreek plant growth parameters such as root and shoot lengths, seedling fresh weights, and leaf count. The use of biofertilizers for the management of kasuri methyl was found to produce substantial growth parameter outcomes [35]. When comparing the development of fenugreek with control plants, Soundari et al. (2015) also showed the noteworthy impacts of biofertilizer inoculation. Vermicompost improves the physical characteristics of the soil, which makes it easier for roots to grow and stimulates the formation of new shoots [36]. It also increases the amount of nutrients that are available to the plant [37]. The findings of Singh et al. (2011) and Mathivanan *et al.*, (2012) demonstrated that the common bean plant's pod length increased with higher vermicompost application [38,39]. The use of vermicompost fertilizer enhanced grain output in fenugreek plants, and grain yield rose as fertilizer level increased [40]. Moreover, combinations of vermicompost organic waste outperformed mixtures of non-vermicompost organic waste in terms of their effect on plant output. The utilization of vermicompost fertilizer increased plant photosynthesis and chlorophyll levels because of its high humic chemical content [41].

Microbial fertilization, combined with Rhizobium and phosphate solubilizing bacteria (PSB), has been shown to improve soil health and crop yield [4]. Applying manures and biofertilizers was found to significantly increase plant height and the quantity of green leaves per plant at the peak stage. In the control group, there were the fewest leaves per plant. Increased leaf count per plant could be the result of improved growth-related characteristics brought about by particular growth-promoting compounds secreted by organic manures, as well as improved water and nutrient uptake and transportation [42]. Fenugreek seed yield is maximized by applying organic amendments in conjunction with dual inoculation of Rhizobium and PSB biofertilizers. It is clear that the biofertilizers have improved the general growth and development of the plants. The biofertilizers have encouraged the growth, development, and yield of fenugreek plants by facilitating the availability of nutrients in the soil and nutrient uptake by the plants [43].

5. Conclusion

The study found that fenugreek seeds treated with T6 (FYM + Azotobacter + PSB) showed increased growth and yield metrics. The study also revealed a greater seed protein content in the northern region. The mix of organic amendments and biofertilizer significantly impacted total plant growth metrics and yield attributing factors. Biofertilizers, including beneficial microorganisms like Rhizobium, Azotobacter, Azospirillum, and PSB, improved overall crop performance in fenugreek by aiding nitrogen fixation, nutrient solubilization, and plant growth. When combined with organic amendments like vermicompost, biofertilizers can enhance the quality of fenugreek, potentially leading to increased nutritional and commercial value. This sustainable and environmentally friendly method of growing fenugreek improves crop quality, productivity, and quality while reducing the adverse environmental effects of chemical fertilizers.

6. References

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