

The Relationship between Vermicompost Biofertilizer and Growing Substrates with *Calendula officinalis* L. Yield and Its Components

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The cultivation of marigold (*Calendula officinalis* L.) has become increasingly significant due to its medicinal benefits and ornamental value. The widespread use of agrochemicals has adversely impacted soil quality, crop yields, and the environment. As a viable alternative, organic amendments and biofertilizers have been suggested to improve both soil and plant health. Vermicompost presents a sustainable approach for providing plant nutrition and enhancing production, thereby promoting soil health and fertility. This experiment was carried out under field conditions to investigate the effects of vermicompost (0, 1, 2, and 3 kg/m²) and growing substrates (field soil, field soil and sand, and field soil, sand, and vermicompost) on some parameters of marigold. Shoot number, flower number, flower diameter, plant dry weight, and flower dry weight were measured. Results showed that the higher levels of vermicompost increased all measured parameters. Comparison between various substrates and different levels of vermicompost showed that substrate including field soil, sand, and animal manure induced more yield and its component than other treatments of the experiment except for 3.0 kg/m² vermicompost. These results underline the potential of bio- and organic fertilizer in improving flower induction of the *C. officinalis* with the possibility of avoiding chemical fertilization.

Abstract

Keywords: Biofertilizers, Cultivation beds, Marigold, Organic fertilizers, Ornamental and medicinal plants.

INTRODUCTION

Marigold (*Calendula officinalis* L.) is a prominent flowering plant belonging to the Asteraceae family, renowned for its vibrant yellow and orange blooms that attract a lot of attention in urban green space, as well as for its medicinal properties (Shaabani *et al.*, 2022). Although, *C. officinalis* is originally from the Mediterranean region, it is cultivated globally, including in countries such as China, Europe, and the United States (Soliman *et al.*, 2024). This species is classified as a winter annual herb. The characteristics of the soil and the environmental conditions significantly influence the quantity and size of the flowers produced each season (Shaabani *et al.*, 2022).

The challenges associated with the overuse of chemical inputs in the agricultural sector, particularly the significant effects on soil pH and the common agricultural production practices, have led to a growing interest in the application of organic and biofertilizer amendments in recent years (Libutti *et al.*, 2020; Shaabani *et al.*, 2022). To achieve optimal yields, it is crucial to improve soil health through the incorporation of organic matter and biofertilizers (Hasan *et al.*, 2024). Although, mineral fertilizers play a vital role, they should be complemented or substituted with accessible organic resources and biofertilizers, especially in light of increasing costs and concerns regarding soil health, environmental sustainability, and human well-being (Hasan *et al.*, 2024). The impact of these amendments on agricultural soils is influenced by various factors, including the characteristics of the feedstock, processing conditions, application rates, soil types, environmental factors, and the specific crop species involved (Urre *et al.*, 2019; Libutti *et al.*, 2020). The adoption of biofertilizers among farmers is rapidly increasing, as these products are effective in enhancing microbial activity, thereby improving nutrient availability for plant uptake (Shaabani *et al.*, 2022). Biofertilizers consist of various combinations of microbes that promote plant growth (Nada *et al.*, 2024). Given that organic amendments and biofertilizers positively influence crop yields without causing environmental harm, their implementation and advocacy represent a viable and promising alternative to chemical fertilizers (Shaabani *et al.*, 2022). Furthermore, organic amendments and biofertilizers contribute to improved plant nutrition, enhanced crop yield and quality, preservation of soil fertility, and the promotion of sustainable agricultural practices (Libutti *et al.*, 2020).

Vermicompost is an organic fertilizer resembling peat, characterized by its high nutritional value, excellent aeration, porosity, and capacity to retain water, produced through the collaborative efforts of earthworms and microorganisms. As an efficient organic fertilizer and biocontrol agent, vermicompost positively influences plant growth, yield, and quality (Joshi *et al.*, 2015). Beyond its role in organic waste management, it is acknowledged as a potent promoter of plant growth (Rehman *et al.*, 2023). The microbial activities within vermicompost enhance the availability of numerous macro- and micronutrients (Rehman *et al.*, 2023). This nutrient-rich fertilizer has gained popularity for the rehabilitation of agricultural soils contaminated with metals (Wang *et al.*, 2018; Zhang *et al.*, 2020). Vermicompost acts as a biofertilizer, comprising a biologically active mix of bacteria, enzymes, plant residues, and earthworm casts that facilitate the decomposition of soil organic matter and enhance microbial activity in the planting medium (Shaabani *et al.*, 2022; Rehman *et al.*, 2023). It is not only environmentally sustainable but also a non-toxic amendment that enriches soil with vital nutrients and growth-enhancing substances. A systematic review conducted by Oyege and Balaji Bhaskar (2023) indicated that incorporating vermicompost into agricultural practices improves soil quality, including increased permeability, aeration, drainage, and water retention, while also enhancing

soil pH and microbial diversity, ultimately leading to higher crop yields. Similar conclusions have been drawn by other researchers (Iqbal *et al.*, 2024; Terefe *et al.*, 2024). Furthermore, vermicompost has demonstrated the ability to convert unavailable nutrients into accessible forms, supplying both micro- and macronutrients to plants, and it has been found to contain higher levels of sulfur (S) compared to mineral fertilizers, which can further promote plant growth (Hoque *et al.*, 2022; Shen *et al.*, 2022; Iqbal *et al.*, 2024). Additionally, vermicompost is more effective than traditional plant compost in lowering heavy metal concentrations in soil and reducing their uptake by plants (Li *et al.*, 2021).

The application of soil amendments, particularly animal manures rich in organic matter and nutrients, serves as a cost-effective approach to enhance both crop yield and soil quality (Antonious *et al.*, 2023). The reprocessing of animal manures can diminish the reliance on synthetic inorganic fertilizers while providing beneficial amendments that improve soil structure and nutrient content. Animal manures are a significant source of ammonia (NH_3), which, upon reacting with water, forms ammonium ions (NH_4^+). These ions readily bind to negatively charged soil organic matter and clay particles, facilitating absorption by plant roots (Antonious *et al.*, 2023). The incorporation of organic amendments, including cattle manure, biochar, and compost, constitutes an environmentally sustainable approach for mitigating heavy metal contamination (Gu *et al.*, 2019; Hamid *et al.*, 2020; Mashur *et al.*, 2021). Nonetheless, these practices are frequently considered impractical due to their costs and the risk of introducing additional pollutants (Pramanik *et al.*, 2018). Organic fertilizers play a crucial role in sustainable agriculture by maintaining nutrient availability, enhancing soil organic matter, and improving the physical and chemical properties of the soil, ultimately contributing to increased crop productivity (Hasan *et al.*, 2024).

Some studies have investigated the impact of vermicompost (Libutti *et al.*, 2020; Shaabani *et al.*, 2022) and animal manure (Antonious *et al.*, 2023; Nada *et al.*, 2024) on the morpho-physiological characteristics and their variations in *C. officinalis*. The application of vermicompost, whether used alone or in combination with other organic amendments, has been shown to enhance growth and yield in some species (Libutti *et al.*, 2020; Makhtoumi *et al.*, 2022; Terefe *et al.*, 2024). The use of biofertilizers resulted in significant improvements in plant height, total biomass yield, seed weight, and harvest index in *C. officinalis*; however, parameters such as flower diameter, number of shoots, dried flower yield, flower number, and seed yield remained unaffected by the treatments (Rezae and Baradaran, 2013). Furthermore, a higher application rate of poultry manure demonstrated notable advantages over chemical fertilizers in terms of plant height, shoot number, plant dry weight, chlorophyll content, carbohydrates, flower number, fresh weight of flower, and carotenoid levels in *C. officinalis* (Nada *et al.*, 2024).

Fertilizer management plays a crucial role in the successful cultivation of marigold plants, and identifying appropriate fertilizers can significantly influence both quantitative and qualitative outcomes (Onofrei *et al.*, 2017). While the impact of fertilizers on crop growth has been thoroughly investigated, research focusing on the response of medicinal herbs to organic fertilizers in Iran remains limited (Shaabani *et al.*, 2022). Most existing studies have been conducted in greenhouse settings, with field studies in open environments being relatively rare. Consequently, this research aimed to examine the effects of varying levels of vermicompost biofertilizer and different growing substrates, particularly animal manure, on the floral characteristics of *C. officinalis*, a valuable ornamental and medicinal plant, in a field setting.

MATERIALS AND METHODS

Plant sample and geographical and climatic coordinates of the experiment site

The seed of ornamental-medicinal *Calendula officinalis* L., from the family Asteraceae, was used as the sample. In order to investigate the effect of different levels of vermicompost biofertilizer and growing substrates on the yield and yield components of this plant in Guilan province, an experiment was conducted in the crop year 2020-2021 at 15 km from Rasht city at an altitude of 70 m above sea level and at a latitude of 37° 10' 5.5" north and longitude 45° 33' 12.6" east. This region has an annual rainfall of 1348 mm and an average annual temperature of 15°C with a humid climate.

Field preparation and plotting

After field preparation and tillage operation, the desired field was divided into 36 plots with dimensions of 1.8 x 3.5 m and an area of 6.3 m² by means of peaking. The distance between experimental plots was 0.5 m and the distance between blocks was considered to be 1 m. Therefore, the total area of the plan, including the buffers (barriers in agricultural lands), was 376.2 m².

Soil characteristics of the experiment site and experimental plan map

In order to prepare the soil sample, 10 one-kg soil samples were prepared from 10 points of the field and from a depth of 0 to 30 cm, and after complete mixing, a mixed sample was transferred to the Water and Soil Laboratory, Islamic Azad University, Rasht Branch for analysis. The result of soil analysis is presented in table 1. The map of the experimental design, having a total of 2 factors, 7 levels, 12 treatments and 36 plots are presented in Fig. 1.

Table 1. The results of the soil test at the experiment site.

Soil depth (cm)	pH	EC (ds/m)	Texture	Organic matter (%)	Potassium (ppm)	Phosphorus (ppm)	Nitrogen (%)
0-30	6.33	0.59	Clay	0.7	84.0	7.0	0.3

R1	F1	F3	F4	F2	F2	F2	F3	F3	F1	F1	F4	F4
	S1	S3	S2	S3	S1	S2	S2	S1	S3	S2	S3	S1
R2	F1	F3	F2	F4	F4	F1	F3	F2	F3	F1	F2	F4
	S3	S1	S2	S2	S1	S1	S3	S3	S2	S2	S1	S3
R3	F1	F4	F2	F4	F3	F2	F3	F2	F1	F4	F1	F3
	S3	S2	S1	S1	S2	S3	S3	S2	S1	S3	S2	S1

Fig. 1. Plan implementation design. F: biofertilizer, S: substrate, and R: replication.

Planting method

By hookah (manual plow of agricultural lands), in each plot, furrow and ridges were created with a distance of 40 cm from each other. After preparing the modified seed, in the required quantity and randomly selecting the experimental plots, it was planted on the ridges with high density. After two weeks, the bushes were thinned.

Irrigation method

After planting the seeds, each plot was irrigated separately by furrow and ridges method. Depending on weather conditions, if effective rainfall did not occur, irrigation was repeated every 8 days. In case of rain, irrigation was done with delay.

To weed

Weeding was done manually in three stages after planting according to different planting dates. The first, second, and third weeding was done 15, 30, and 45 days after planting, respectively.

Measurement of parameters

To measure the number of shoots in each plant, in each experimental plot, 5 plants were selected and the number of shoots in them was counted. To measure the number of flowers per plant, 5 plants were selected in each experimental plot and the number of flowers produced in them was counted. To measure the flower diameter, it was measured with a digital caliper after the flowers bloomed and before they were harvested. The average diameter of flowers in each sample was calculated as flower diameter. To measure the dry weight of the plant, in each experimental plot, 5 plants were selected and cut from the collar. After weighing each plant separately, drying them in an oven at 72°C for 6 h. Finally, the average dry weight of the plant was recorded in each plot. To measure the dry weight of flowers, 5 flowers were selected in each experimental plot and dried in an oven with a temperature of 45°C for 6 h. Finally, the average flower dry weight was recorded as flower yield per plant.

Experimental design and data analysis

A split plot experiment with a randomized complete block design (RCBD) was used in three replications. Vermicompost biofertilizer at three levels of 0, 1, 2, and 3 kg/m² (added to the farm soil), as the first factor, and substrate growing including farm soil, farm soil combined with sand (in ratio of 1:1), and farm soil combined with sand and animal manure (in ratio of 1:1:1) were used as the second factor. The significance of differences between mean values was calculated using LSD test performed at $P < 0.05$. All data were presented as means \pm SE for at least three replications for each sample. The statistical analysis and means comparison were done using SPSS Statistics software.

RESULTS

There was significant difference (at $P < 0.01$) in all measured parameters (shoot number, flower number, flower diameter, plant dry weight, and flower dry weight) among the seven individually treatments when analyzed by an ANOVA test (Table 2). Statistically significant difference was not observed in the amount of all measured parameter during interaction effect of different levels of vermicompost and various substrates (Table 2).

Table 2. Analysis of variance of the effect of different levels of vermicompost biofertilizer and different growing substrates on *Calendula officinalis* L. yield and its components.

S.o.V	df	MS				
		Shoot number per plant	Flower number per plant	Flower diameter	Plant dry weight	Flower dry weight
Replication	2	0.65	2533.55	0.69	4.90	0.27
Substrates (A)	2	1.87**	271.86**	2.55**	582.13**	9.44**
Biofertilizer (B)	3	2.75**	38.63 ^{ns}	2.07**	635.56**	9.66**
A \times B	6	0.15 ^{ns}	10.09 ^{ns}	0.14 ^{ns}	75.13 ^{ns}	0.66 ^{ns}
Error	18	0.24	43.12	0.33	30.01	0.57
CV (%)	-	8.24	18.58	13.28	9.80	9.89

** and ns: Significant at $P < 0.01$ and insignificant based on the LSD test, respectively. df: degree of freedom, CV: coefficient of variations.

Shoot number

The highest number of shoot per plant (6.50) was obtained in the plants produced from the seeds grown in the substrate containing 3 kg/m² of vermicompost. The difference in the number of shoots obtained in this treatment with the number of shoots obtained in substrate containing field soil together with sand and animal manure (with 6.35 shoots), 2 kg/m² of vermicompost (with 6.15 shoots), and 1 kg/m² of vermicompost (with 6.03 shoots), was not significant. The lowest number of shoots per plant (5.15) was obtained in plants produced from seeds grown in the substrate without vermicompost (Table 3).

Table 3. Mean comparison of the effect of different levels of vermicompost biofertilizer and different growing substrates on *Calendula officinalis* L. yield and its components.

Treatments	Shoot number per plant	Flower number per plant	Flower number per plant	Plant dry weight (g)	Flower dry weight (g)
Vermicompost (kg/m²)					
0.0	5.19 ^b ± 0.13	22.60 ^c ± 2.06	22.60 ^c ± 2.06	46.55 ^d ± 5.28	6.36 ^c ± 1.03
1.0	6.03 ^a ± 0.32	22.74 ^c ± 1.80	22.74 ^c ± 1.80	52.92 ^c ± 4.75	7.41 ^b ± 1.33
2.0	6.15 ^a ± 1.02	24.38 ^b ± 1.57	24.38 ^b ± 1.57	57.51 ^b ± 6.20	7.85 ^b ± 1.24
3.0	6.50 ^a ± 1.21	27.60 ^a ± 1.78	27.60 ^a ± 1.78	66.53 ^a ± 5.04	8.86 ^a ± 1.14
Substrates					
Field soil	5.56 ^c ± 0.63	18.74 ^b ± 3.10	18.74 ^b ± 3.10	47.98 ^c ± 4.68	6.63 ^c ± 1.32
Field soil + sand (1:1)	5.99 ^b ± 0.82	26.20 ^{ab} ± 1.54	26.20 ^{ab} ± 1.54	58.53 ^b ± 5.55	7.90 ^b ± 1.19
Field soil + sand + animal manure (1:1:1)	6.35 ^a ± 0.39	27.60 ^a ± 1.84	27.60 ^a ± 1.84	61.13 ^a ± 4.73	8.33 ^a ± 1.05

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Flower number

The plants obtained from the seeds grown in the substrate containing 3 kg/m² of vermicompost and substrate field soil together with sand and animal manure produced the highest number of flowers per plant (27.60). The difference in the number of flower obtained in these two treatments with the number of flower obtained in the other treatments was significant. The lowest number of flowers per plant (18.74) was obtained in the plants produced from the seeds grown in the field soil substrate (Table 3).

Flower diameter

The flowers produced in the plants obtained from the seeds grown in the substrate containing 3 kg/m² of vermicompost and field soil together with sand and animal manure had the largest diameter (4.63 cm). The difference between the diameter of the flower obtained in this treatment and the diameter of the flowers obtained in the treatments of 2 and 1 kg/m² of vermicompost (with diameters of 4.61 and 40.4 cm, respectively) was not significant. The smallest flower diameter (with 3.61 and 3.80 cm) was calculated in the plants obtained from the seeds grown in the substrate without vermicompost and the field soil substrate, respectively (Table 3).

Plant dry weight

Table 3 shows that the highest dry weight of the plant (66.53 g) was calculated by weighing the plants obtained from the seeds grown in the substrate containing 3 kg/m² of vermicompost. The difference between the dry weight of the plant obtained in this treatment and the dry weight of the plant obtained in the treatment of field soil together with sand and animal manure (with 61.13 g) was not significant. The lowest dry weight of the plant (with 46.55 and 47.98 g) was obtained in the plants produced from the seeds grown in the substrate without vermicompost and the field soil substrate, respectively (Table 3).

Flower dry weight

The data obtained from the mean comparison (Table 3) revealed that the highest dry weight of the flower (8.86 g) was obtained by weighing the flowers produced in the plants from the seeds grown in the substrate containing 3 kg/m² of vermicompost. The difference in the dry weight of the flower obtained in this treatment with the dry weight of the flower obtained in the field soil together with sand and animal manure (with 8.33 g) was not significant. The lowest dry weight of flowers (with 6.36 and 6.63 g) was obtained from the weighing of flowers produced in plants from seeds grown in vermicompost-free substrate and field soil substrate, respectively (Table 3).

DISCUSSION

Marigold (*Calendula officinalis* L.) is a notable flowering plant that garners significant interest in urban green spaces, primarily due to its vibrant yellow and orange blooms. A key objective within the floriculture sector, particularly concerning ornamental flowers, is to enhance both the quantity and size of these flowers. The current study revealed that the application of 3 kg/m² of vermicompost, as well as a substrate composed of field soil mixed with sand and animal manure, resulted in an increase in both the number and size of flowers in marigold.

In marigold cultivation, the application of vermicompost biofertilizer and organic fertilizers, such as animal waste, has been demonstrated to significantly enhance both plant yield and its components (Khalid and da Silva, 2012; Rezae and Baradaran, 2013; Shaabani *et al.*, 2022; Nada *et al.*, 2024). The combination of vermicompost with humic acid as an organic fertilizer has been shown to increase the number of flowers produced in marigold (Shaabani *et al.*, 2022). Our research indicated that both vermicompost and animal manure, when applied individually, resulted in an increase in the number and size of flowers. The incorporation of organic amendments into the soil, particularly those derived from animal waste (Mashur *et al.*, 2021), as well as vermicompost (Makhtoumi *et al.*, 2022; Hoque *et al.*, 2022; Shen *et al.*, 2022; Oyege and Balaji Bhaskar, 2023; Iqbal *et al.*, 2024; Terefe *et al.*, 2024; Rehman *et al.*, 2023), and their combined use (Libutti *et al.*, 2020; Antonious *et al.*, 2023; Hasan *et al.*, 2024), has been associated with enhanced growth and yield in various other plant species.

The impact of vermicompost on the growth and flowering of marigolds in greenhouse conditions was assessed (Sardoei, 2014). The application of vermicompost fertilizer was found to have the most significant influence on the biomass of basil (*Ocimum basilicum* L.) (Makhtoumi *et al.*, 2022). According to Sultana *et al.* (2015), the use of vermicompost enhanced various growth and flowering metrics of *Zinnia elegans*, including shoot and root lengths, leaf number, flower number, flower diameter, and both fresh and dry weights of flowers, in comparison to NPK fertilizer (Nada *et al.*, 2024). In the case of *Foeniculum vulgare*, the application of biofertilizers, especially a mixture of bacteria and fungi, led to increases in total plant fresh weight, dry weight, and shoot numbers when compared to the control group

(Nada *et al.*, 2022). In alignment with our findings, it was noted that the use of organic and biofertilizers significantly improved the number and diameter of flowering heads in marigold plants compared to those that were not fertilized (EL-Zawawy *et al.*, 2021). A higher level of organic fertilization was associated with the greatest flower number and yield (Nada *et al.*, 2024). A meta-analysis indicated that the incorporation of vermicompost into the soil markedly enhances shoot biomass in plants (van Groenigen *et al.*, 2014). The beneficial effects of vermicompost on various medicinal plants, including garlic (*Allium sativum* L.) (Verma *et al.*, 2013), coriander (*Coriandrum sativum* L.) (Godara *et al.*, 2014), German chamomile (*Matricaria chamomilla* L.) (Ansarifar *et al.*, 2012), and basil (*Ocimum basilicum*) (Sirousmehr *et al.*, 2014), have been documented. Experimental results demonstrated that the application of vermicompost significantly improved growth parameters such as shoot biomass and flower quantity in African marigold compared to mineral fertilizers (Joshi *et al.*, 2015).

The utilization of animal manure as an organic fertilizer possesses significant characteristics that are unattainable through synthetic inorganic fertilizers. The microorganisms present in animal manures promote the gradual release of the primary plant nutrients—nitrogen (N), phosphorus (P), and potassium (K)—from soil organic matter, thereby minimizing their offsite movement into natural water bodies and mitigating the risk of eutrophication (Antonious *et al.*, 2023). In contrast to our findings, previous research indicated that the application of poultry manure had a more substantial effect on marigold growth than biofertilizers (Nada *et al.*, 2024). The results of this study indicated that the application of organic fertilizers and plant growth-promoting microbes had a beneficial impact on the growth and flowering of marigold when compared to the control treatment.

The literature indicates that the impact of incorporating organic fertilizers into cultivated soil on crop growth exhibits considerable variability and inconsistency. This variability may originate from factors such as the types of fertilizer feedstock, pyrolysis conditions, the structure and composition of the fertilizers, soil characteristics, and the specific crops being tested (Meschewski *et al.*, 2019; Libutti *et al.*, 2020). The effective provision of N through biological fertilizers, particularly vermicompost, which is rich in biologically active substances that function as growth regulators, can elucidate the positive effects of vermicompost on both vegetative and reproductive growth (Makhtoumi *et al.*, 2022). The application of biological fertilizers to medicinal plants such as chamomile (*Matricaria chamomilla* L.) and marigold has been shown to enhance flower yield (Makhtoumi *et al.*, 2022). Furthermore, when organic materials are introduced into the soil, they improve its physical properties, including aeration, aggregation, permeability, and water retention capacity (Yadav *et al.*, 2023), all of which are conducive to plant growth and development (Nada *et al.*, 2024). The ability of organic manure to retain moisture and maintain adequate pore spaces for effective air circulation and drainage of excess water may contribute to its beneficial effects on shoot numbers and plant dry weight (Nada *et al.*, 2024). It is likely that the application of suitable quantities of vermicompost enhances soil microbial activity and the production of plant growth regulators by these microorganisms, leading to improved nutrient absorption, increased photosynthesis, and greater dry matter accumulation, ultimately resulting in enhanced flowering (Rezae and Baradaran, 2013).

CONCLUSION

It is crucial to develop strategies that improve both the yield and the components contributing to the yield of ornamental-medicinal plants. Research highlights the potential of bio- and organic fertilizers in promoting flower induction in the *C. officinalis* species, which

may facilitate a reduction or complete removal of synthetic and chemical fertilizers, thereby fostering a more sustainable and ecologically sound agricultural framework. The incorporation of vermicompost and organic fertilizers into the soil likely enhances the availability of essential nutrients for the plants, improves the physical and chemical properties of the soil, aids in N fixation, promotes plant growth regulators, produces antibiotics, and facilitates the decomposition of organic matter, all while creating an optimal environment for root development. This, in turn, contributes to the growth and development of the plant's aerial parts. Additional studies are necessary to clarify the specific mechanisms by which organic and biofertilizers affect the growth and flowering of marigolds. Gaining insight into these mechanisms will assist in optimizing the application of these interventions to maximize their advantages. Future research that integrates biological and organic fertilizers is expected to further enhance flowering in marigold plants. The findings suggest that the application of biological and organic amendments to the soil can improve both the quantitative and qualitative characteristics of marigold. However, the varied responses of plants to these fertilizers indicate the need for further experimentation.

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