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THE EFFECT OF BIOFERTILIZER AND AGRICULTURAL SULFUR LEVELS ON THE GROWTH AND YIELD AND QUALITY OF BROCCOLI

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ABSTRACT

A field experiment was conducted in an experimental field affiliated with the College of Agricultural Engineering Sciences, University of Baghdad, Al-Jadriya, during the fall season of 2022. Split plot arrangement in the Randomized Complete Block Design (RCBD) of three replicates was used comprising three levels of sulfur (0, 500, and 1000 kg ha^{-1}) symbolized by S_0, S_1, and S_2 forming the main plots, added to the soil before planting before two months, while the subplots were the bio-fertilizers represented by B_0 and B_1. Results showed the superiority of the second level of agricultural sulfur, giving the highest traits involving the plant height reaching 79.2 cm, chlorophyll content that was 91.1 SPAD, total yield was 44.0 mg ha^{-1} and protein content was 18.38%. The biofertilization was also superior in giving the highest traits involving the plant height was 80.2 cm, chlorophyll content was 98.9 SPAD and flower disc diameter was 23. 53 cm, total yield was 43.6 mg ha^{-1} and protein content was 18.94%, the concentration of the active substance sulforaphane in flower disc was 38.1 mg kg^{-1} fresh weight. For the binary interaction, the treatment of biofertilization with the third level of agricultural sulfur was superior, giving the highest values for the qualities in the plant height was 81.5 cm and the flower disc diameter was 23. 92 cm and the total yield was 45.7 mg ha^{-1}, the percentage of protein in the amount of 19.31% and the concentration of the active substance sulforaphane in flower disk was 47.8 mg kg^{-1} fresh weight.

Keywords: Agricultural sulfur, Biofertilizers, Broccoli.

تأثير اللقاح الحيوي ومستويات الكبريت الزراعي في نمو وحاصل ونوعية نبات البروكلي

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الخلاصة

نفذت تجربة حقلية خلال الموسم الخريفي 2022 في احد حقول التجارب التابعة لكلية علوم الهندسة الزراعية- جامعة بغداد -الجادرية. باستعمال ترتيب الالواح المنشقة بتصميم القطاعات العشوائية الكاملة (RCBD) وبثلاثة مكررات الرئيسية لإضافة الكبريت الزراعي بمستويات (0 و500 و1000) كغم هـ$^{-1}$ بالتتابع والتي اضيفت قبل الزراعة بشهرين ويرمز له بالرمز(S_0, S_1, S_2) بينما تمثل الالواح الثانوية اضافة السماد الحيوي ويعبر عنه بالرمز(B_0, B_1). اظهرت النتائج تفوق المستوى الثاني للكبريت الزراعي واعطى اعلى القيم لصفات في ارتفاع النبات بقيمة بلغت 79.2 سم وفي محتوى الكلوروفيل للأوراق SPAD 91.1 وفي الحاصل الكلي بقيمة بلغت 44.0 ميكاغرام هـ$^{-1}$ وفي نسبة البروتين بقيمة

*The article is taken from the master's thesis of the first researcher.

بلغت 18.3 % .كما تفوق التلقيح الحيوي واعطى اعلى القيم لصفات في ارتفاع النبات بقيمة بلغت 80.2 سم وفي محتوى الكلوروفيل للأوراق SPAD 98.9 وفي قطر الاقراص الزهرية بقيمة بلغت 23.53 سم وفي الحاصل الكلي بقيمة بلغت 43.6 ميكاغرام هـ$^{-1}$ وفي نسبة البروتين بقيمة بلغت 18.94%، وفي تركيز المادة الفعالة السالفورافان في الاقراص الزهرية بقيمة بلغت 38.1 ملغم كغم $^{-1}$ وزن رطب. اما التداخل الثنائي فقد تفوق معاملة التلقيح الحيوي مع المستوى الثالث للكبريت الزراعي واعطت اعلى القيم للصفات في ارتفاع النبات بقيمة بلغت 81.5 سم وفي قطر القرص بقيمة بلغت 92. 23 سم وفي الحاصل الكلي بقيمة بلغت 7 .45 ميكاغرام هـ $^{-1}$ وفي نسبة البروتين بقيمة بلغت 19.31% وفي تركيز المادة الفعالة السالفورافان في الاقراص الزهرية بقيمة بلغت 47.8 ملغم كغم $^{-1}$ وزن رطب.

الكلمات المفتاحية: الكبريت الزراعي، لقاح حيوي، البروكلي.

INTRODUCTION

Sulfur is one of the major nutrients essential for plant nourishment. Its content in plants ranges from 0.1 to 1%. Deficiency symptoms appear on the plant when the sulfur content decreases to less than 0.1%. Sulfur movement within plants is slow, and plants absorb sulfur as sulfate SO_4^{-2} ions form. As for atmospheric sulfur, SO_2, and H_2S, plants can take up it through the stomata, and then it is assimilated within the plant in the form of amino acids involved in proteins' synthesis, including Systine and Cysteine where sulfur constituent 2.7% S, as well as Methionine, containing a sulfur percentage of 21% (**Havlin *et al*, 2005**). The addition of sulfur affects the properties of the soil and is considered a repairer of calcareous soils, by reducing the degree of soil interaction and increasing nutrient availability (**EL- Fahdawi *et al*, 2020; Jafaar & Abdul rsool, 2023**). Bio-fertilizers have a significant role in conserving soil fertility for a long extent. They are considered a renewable source of the required nutrients that plants need for proper growth as the microorganisms transform the complex or unavailable elements found in the soil into available and absorbable by the plant (**Abdul- Hassein & Hassan, 2021; Shaker & AL- Bahrani, 2022; Sabry& Abdal - latife, 2017**). *Thiobacillus* is a small single or double bacillus bacteria that is straight, gram-negative, mobile by flagella and does not form internal spores. It is a Chemolithotroph that derives energy from oxidizing sulfur compounds in well-ventilated soil into sulfuric acid (**Robertson & Kuenen, 2006**). Broccoli is a cold-season crop grown year-round in cold regions and needs a moderate climate that tends to be warm during its vegetative growth stages. Broccoli has a great interest in the medical research community, as many previous medical studies indicated that there is a positive role for eating broccoli in preventing cancer diseases effectively due to its high content of Sulforaphane, as well as some other Glucosinolates derivatives. Scientific studies have shown not only the ability of Sulforaphane to reduce the activity of cancer cells but also showed its selective ability to carry out the process of targeting and selective destruction of cancer cells without affecting healthy cells in the human body because it is a good source of natural antioxidants (**Renuka & Thangam, 2010; Ali *et al*, 2016; Wahed & AL- AZawi, 2023).**

MATERIALS AND METHODS

The experiment was conducted at an expermintal field affiliated to the College of Agricultural Engineering Sciences, University of Baghdad, Al-Jadriya, during the fall season 2022. The soil preparations, involving tillage practices and land dividing, were carried out; next, the seedlings were planted in rows lengthened 3m separated by 75cm with a distance of 45cm between pits. Split plot order within the Randomized Complete Block Design (RCBD) of three replicates was used where the main plots involved the agricultural sulfur at three levels (0, 500, and 1000 kg ha^{-1}), added two months before planting, referred to as S_0, S_1, and S_2,

respectively, while the subplots included the bio-fertilizer symbolized by B_0 and B_1 referring to the seedling root contaminated and non-contaminated respectively. Thus, the experimental units were 18 (3x2x3). The biofertilizer *Thiobacillus* was added before planting the seedlings, the bacterial vaccine was placed in a clean container, and Arabic gum was placed at a concentration of 20%, as broccoli seedlings were placed in Arabic gum, then the seedlings were dipped in the biovaccine several times and for half an hour and placed on a clean cloth for the purpose of drying, after that the seedlings were planted in the field. The experimental soil was fertilized with 80 kg ha^{-1} of urea (46%N) added in two batches, 120 kg $P.ha^{-1}$ as Diammonium phosphate, and 150 kg $K.ha^{-1}$ as Potassium sulfate (41.5 % K). The irrigation method was done by drip irrigation. Soil samples were taken from the field from several regions at 0-30 cm depth before planting. They were air-dried, grinding with a hammer, and passed through a sieve with a diameter of 2 mm (Table 1) to analyze the physical and chemical characteristics of the soil experimental field before planting.

Table (1): Some physical and fertility properties of the soil under studying.

Soluble cations and anions Mmole L^{-1}							Soil textue	O.M (%)	Available mg kg^{-1}soil			EC $dS.m^{-1}$	pH
CO_3^{2-}	HCO^{-3}	Cl^-	SO_4^{2-}	Na^+	Mg^{2+}	Ca^{2+}			K	P	N		
Nill	2.9	4	13.7	1.82	6.9	11.9	Loam	2.1	78	18.8	38.0	2.1	7.8

RESULTS AND DISCUSSION

1-Plant height (cm)

Results demonstrated in Table 2 that the effect of the bio-fertilization with *Thiobacillus* was significant on the plant height, as the treatment B_1 was superior, producing 80.2 cm on average compared to the B_0, which gave the lowest value averaging 73.9 cm, achieving an increase of 8.52%. The agricultural sulfur at level S_1 was significantly superior, producing the highest average of plant height reaching 79.2 cm. In comparison, the lowest average was 73.9 cm, given by level S_0 with an increase of 7.17%. The binary interaction between the bio-fertilization and the agricultural sulfur levels affected the plant height resulting in significant differences between the interaction treatments. Table 2 shows that the treatment S_2B_1 gave the highest average, plant height reaching 81.5 cm, superiorly higher than the lowest plant height recorded by S_0B_0, which gave 69.1 cm by 17. 94%.

Table (2): Effect of the bio-fertilization and agricultural sulfur levels on the plant height (cm).

Mean B	Sulfur Level			Bio fertilization
	S2	S1	S0	
73.9	74.8	77.9	69.1	B0
80.2	81.5	80.6	78.7	B1
4.92	8.53			LSD
	78.1	79.2	73.9	Mean S
	5.03			LSD

2- Stem diameter (mm)

Results demonstrated in Table 3 that the effect of the bio-fertilization with *Thiobacillus* was significant on the Stem diameter, as the treatment B_1 was superior, producing 30.49 mm on average compared to the B_0, which gave the lowest value averaging 24.81 mm, achieving an increase of 22.89 %. The agricultural sulfur at level S_2 was significantly superior, producing the highest average of Stem diameter reaching 30.62 mm. In comparison, the lowest average was 23.71 mm, given by level S_0 with an increase of 29.14%. The binary interaction between the bio-fertilization and the agricultural sulfur levels affected the Stem diameter resulting in significant differences between the interaction treatments. Table 3 shows that the treatment S_1B_1 gave the highest average Stem diameter, reaching 31.65 mm, superiorly higher than the lowest Stem diameter recorded by S_0B_0, which gave 18.58 mm with an increase 70. 34%.

Table (3): Effect of the bio-fertilization and agricultural sulfur levels on the Stem diameter (mm).

Mean B	Sulfur Level			Bio fertilization
	S2	S1	S0	
24.81	30.25	25.58	18.58	B0
30.49	31.00	31.65	28.83	B1
3.08	5.33			LSD
	30.62	28.62	23.71	Mean S
	3.77			LSD

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3 Dry weight of leaves (g plant $^{-1}$)

Results demonstrated in Table 4 that the effect of the bio-fertilization with *Thiobacillus* was significant on the Dry weight of leaves, as the treat ment B_1 was superior, producing 249 g plant $^{-1}$ on average compared to the B_0, which gave the lowest value averaging 173 g plant $^{-1}$, achieving an increase of 43. 93 %. The agricultural sulfur at level S_1 was significantly superior, producing the highest average of Dry weight of leaves reaching 253 g plant $^{-1}$. In comparison, the lowest average was 191 g plant $^{-1}$, given by level S_0 with an increase of 32.46%. The binary interaction between the bio-fertilization and the agricultural sulfur levels affected the Dry weight of leaves resulting in significant differences between the interaction treatments. Table 4 shows that the treatment S_1B_1 gave the highest average Dry weight of leaves, reaching 309 g plant $^{-1}$, superiorly higher than the lowest Dry weight of leaves recorded by S_0B_0, which gave 143 g plant $^{-1}$ with an increase 116. 08%.

Table 4. Effect of the bio-fertilization and agricultural sulfur levels on the Dry weight of leaves (g plant $^{-1}$).

Mean B	Sulfur Level			Bio fertilization
	S2	S1	S0	
173	176	198	143	B0
249	201	309	238	B1
57.8	117.5			LSD
	189	253	191	Mean S
	60.1			LSD

4 - Flower disc diameter (cm)

Results demonstrated in Table 5 that the effect of the bio-fertilization with *Thiobacillus* was significant on the flower disc diameter, as the treatment B_1 was superior, producing 23.53 cm on average compared to the B_0, which gave the lowest value averaging 20.83 cm, achieving an increase of 12.96 %. The agricultural sulfur at level S_2 was significantly superior, producing the highest average of flower disc diameter reaching 23.33 cm. In comparison, the lowest average was 20.04 cm, given by level S_0 with an increase of 16.41%. The binary interaction between the bio-fertilization and the agricultural sulfur levels affected the flower disc diameter resulting in significant differences between the interaction treatments. Table 5 shows that the treatment S_2B_1 gave the highest average, flower disc diameter reaching 23.92 cm, superiorly higher than the lowest flower disc diameter recorded by S_0B_0, which gave 16.50 cm with an increase 44. 96%.

Table (5): Effect of the bio-fertilization and agricultural sulfur levels on the flower disc diameter (cm).

Mean B	Sulfur Level			Bio fertilization
	S2	S1	S0	
20.83	22.75	23.25	16.50	B0
23.53	23.92	23.08	23.58	B1
1.79	3.10			LSD
	23.33	23.17	20.04	Mean S
	2.19			LSD

5- flower disc weight (g plant $^{-1}$)

Results demonstrated in Table 6 that the effect of the bio-fertilization with *Thiobacillus* was significant on the flower disc weight, as the treatment B_1 was superior, producing 1063 g plant $^{-1}$ on average compared to the B_0, which gave the lowest value averaging 790 g plant $^{-1}$, achieving an increase of 34. 55 %. The agricultural sulfur at level S_1 was significantly superior, producing the highest average of flower disc weight reaching 1047 g plant $^{-1}$. In comparison, the lowest average was 851 g plant $^{-1}$, given by level S_0 with an increase of 21.85%. The binary interaction between the bio-fertilization and the agricultural sulfur levels affected the flower disc weight of leaves resulting in significant differences between the interaction treatments. Table 6 shows that the treatment S_1B_1 gave the highest average flower disc weight, reaching 1132 g plant $^{-1}$, superiorly higher than the lowest flower disc weight recorded by S_0B_0, which gave756 g plant $^{-1}$ with an increase 49. 73%.

Table (6): Effect of the bio-fertilization and agricultural sulfur levels on the flower disc weight of leaves (g plant $^{-1}$).

Mean B	Sulfur Level			Bio fertilization
	S2	S1	S0	
790	674	942	756	B0
1063	1112	1132	945	B1
134.3	293.1			LSD
	893	1037	851	Mean S
	173.2			LSD

6- Total yield of broccoli (mg ha^{-1})

Results demonstrated in Table 7 that the effect of the bio-fertilization with *Thiobacillus* was significant on the broccoli total yield, as the treatment B_1 was superior, producing 43.6 mg ha^{-1} on average compared to the B_0, which gave the lowest value averaging 38.9 mg ha^{-1}, achieving an increase of 12.08%. The agricultural sulfur at level S_1 was significantly superior, producing the highest average of the broccoli total yield reaching 44.0 mg ha^{-1}. In comparison, the lowest average was 38.2 mg ha^{-1}, given by level S_0 with an increase of 15.18%. The binary interaction between the bio-fertilization and the agricultural sulfur levels affected the total yield resulting in significant differences between the interaction treatments. Table 7 shows that the treatment S_2B_1 gave the highest average broccoli total yield, reaching 45.7 mg ha^{-1}, superiorly higher than the lowest broccoli yield recorded by S_0B_0, which gave 36.1 mg ha^{-1} with an increase 26.59%.

The reason for the significant increase in the studied characteristics can be attributed shown in the tables aboveto the role the microorganisms have the ability to enhance plant growth under normal and harsh conditions through various direct and indirect mechanisms, including the processes of nitrogen fixation, phosphate and potash dissolving, and production of growth regulators, hormones, and water hydrolyzed enzymes, and hence providing nutrients sufficiently to obtain high productivity without disturbing the balance. Furthermore, microorganisms organize different processes that may occur in the soil, including the organic matter decomposition and access to the nutrient elements essential for plant growth leading to an increase in the yield. This is due to why microbiology is effective in increasing nutrient availability as well as stimulating the production of certain phytohormones (**Kour *et al*, 2020; Al-Obaidi & Abdul -Ratha, 2021; Al-Silmawy & Abdul -Ratha, 2023; AL - Rubaye *et al*, 2019**; **AL-mamori & Abdul -Ratha , 2020).** Especially sulfur-oxidizing bacteria, which in turn increase the availability of sulfur in the soil, and this indicates that the higher the concentration of sulfur in the soil, it works to reduce the pH of the soil, increase the availability of nutrients in it, and produce a good root system for the plant, and its absorption by the plant, especially nitrogen, which leads to increased vegetative growth and the dry weight gain of the leaves (**Maruf & Tahir, 2020**) as well as the important cycle in the vital processes in the plant and that sulfur works to stimulate and divide plant cells and their growth In plant height, stem diameter, flower disc diameter and flower disc weight (**Muthanna *et al*, 2017**) according to **Ali *et al*. (2014**). as sulfur has contributed to increasing the content of the plant one of the basic components of the amino acid tryptophane, which enters the composition of oxins, including the Indole Acetic Acid (IAA) responsible for the height of the plant (**AL–Hasson,2010**; **Ali *et al*, 2014**). Sulfur a positive role in many physiological functions, including the synthesis of amino acids and an increase in the spread of plant roots in the soil, which leads to an increase in the rate of photosynthesis, which is positively reflected in the increase in the qualities of the total yield.**(Cattoo *et al*, 2018; Naser & AL - Tameemi, 2020)** and these results agree with **Patidar *et al*. (2017)**.

Table (7): Effect of the bio-fertilization and agricultural sulfur levels on the total yield (mg ha^{-1}).

Mean B	Sulfur Level			Bio fertilization
	S2	S1	S0	
38.9	37.3	43.2	36.1	B0
43.6	45.7	44.9	40.3	B1
7.94	13.76			LSD
	41.5	44.0	38.2	Mean S
	9.73			LSD

7 - Chlorophyll content of leaves (SPAD)

Results demonstrated in Table 8 that the effect of the bio-fertilization with *Thiobacillus* was significant on the chlorophyll content, as the treat ment B_1 was superior, producing 98.9 SPAD on average compared to the B_0, which gave the lowest value averaging 69.2 SPAD, achieving an increase of 42. 91 %. The agricultural sulfur at level S_1 was significantly superior, producing the highest average of chlorophyll content of leaves reaching 91.1 SPAD. In com parison, the lowest average was 80.0 SPAD, given by level S_0 with an increase of 13.87%. The binary interaction between the bio-fertilization and the agricultural sulfur levels affected the Dry weight of leaves resulting in significant differences between the interaction treatments. Table 8 shows that the treatment S_1B_1 gave the highest average chlorophyll content of leaves, reaching 114.0 SPAD, superiorly higher than the lowest chlorophyll content of leaves recorded by S_0B_0, which gave 65.6 SPAD with an increase 73. 78%. This is due to the higher the rate of sulfur addition, the greater the percentage of chlorophyll in the leaves, and also the higher the rate of sulfur addition, the greater the availability of nutrients in the soil, which increases the chlorophyll content in the leaves, according to **AL–Hasson (2010); Kadhem (2016); Al-Gharani (2022**).

Table (8): Effect of the bio-fertilization and agricultural sulfur levels on the chlorophyll content of leaves (SPAD).

Mean B	Sulfur Level			Bio fertilization
	S2	S1	S0	
69.2	73.9	68.2	65.6	B0
98.9	88.2	114.0	94.5	B1
7.54	16.52			LSD
	81.0	91.1	80.0	Mean S
	9.68			LSD

8 - Protein ratio (%)

Results demonstrated in Table 9 that the effect of the bio-fertilization with *Thiobacillus* was significant on the protein ratio, as the treatment B_1 was superior, producing 18.94 % on average compared to the B_0, which gave the lowest value averaging 16.16%, achieving an increase of 17.20%. The agricultural sulfur at level S_1 was significantly superior, producing the highest average of protein ratio reaching 18.38%. In comparison, the lowest average was 15.99%, given by level S_0 with an increase of 14.94%. The binary interaction between the bio-fertilization and the agricultural sulfur levels affected the protein ratio of leaves resulting in

significant differences between the interaction treatments. Table 9 shows that the treatment S_2B_1 gave the highest average protein ratio, reaching 19.31%, superiorly higher than the lowest protein ratio recorded by S_0B_0 which gave 13.39% with an increase of 44.21%. This is due to the increase in the activity of *Thiobacillus* bacteria and reduce the degree of soil interaction and increase the availability of nutrients in the soil, and increase their absorption by the plant and the greater the rate of addition of sulfur concentrations leads to a significant effect in increasing the proportion of protein in the flower disc, and the reason for this is that sulfur has a role in the) formation of protein through the formation of amino acids (Cystiene- Systine- Methionin) containing sulfur in the plant, and they exist in the form of free acids and also work as building blocks in the construction of Protein (**Kadhem,2016; Maruf & Tahir, 2020**).

Table (9): Effect of the bio-fertilization and agricultural sulfur levels on the protein ratio (%).

Mean B	Sulfur Level			Bio fertilization
	S2	S1	S0	
16.16	17.29	17.81	13.39	B0
18.94	19.31	18.95	18.58	B1
1.173	2.031			LSD
	18.30	18.38	15.99	Mean S
	1.436			LSD

9- Concentration of the active substance sulforaphane in flower disc (mg kg^{-1}fresh weight)

Results demonstrated in Table 10 that the effect of the bio-fertilization with *Thiobacillus* was significant on the concentration of the active substance sulforaphane in flower disc, as the treatment B_1 was superior, producing 38.1 mg.kg^{-1} fresh weight on average compared to the B_0, which gave the lowest value averaging 30.9 mg.kg^{-1}fresh weight, achieving an increase of 23. 30 %. The agricultural sulfur at level S_2 was significantly superior, producing the highest average of concentration of the active substance sulforaphane in flower disc, reaching 43.1 mg kg^{-1} freah weight. In comparison, the lowest average was 22.1 mg kg^{-1} fresh weight, given by level S_0 with an increase of 95.02%. The binary interaction between the bio-fertilization and the agricultural sulfur levels affected the concentration of the active substance sulforaphane in flower disc of leaves resulting in significant differences between the interaction treatments. Table 10 shows that the treatment S_2B_1 gave the highest average concentration of the active substance sulforaphane in curds, reaching 47.8 mg kg^{-1}fresh weight, superiorly higher than the lowest concentration of the active substance sulforaphane in flower disc recorded by S_0B_0, which gave 19.3 mg kg^{-1} fresh weight with an increase of 147.66 %. The increase in the compound of salforaphane in the flower disc, is attributed to the role of agricultural sulfur, as the more sulfur is added, the greater the activity of bacteria, the lower the degree of soil interaction and increases the availability of nutrients, which facilitates the plant to absorb them and thus increase their concentration within the plant (**Kadhem, 2016**). and that all these factors have an impact on the biosynthesis of compounds, especially antioxidants in the flower disc of broccoli, as well as perhaps also the increase in the concentration of the active ingredient sulforaphane is due to the role of fertilizer This is reflected in increasing their percentage in plants, especially nitrogen, phosphorus, potassium and calcium, which effectively contributes to the increase in antioxidants (**Jasim &Al- Timmen, 2014)** these findings are consistent with **Naguib *et al* (2012**).

Table (10): Effect of the bio-fertilization and agricultural sulfur levels on the concentration of the active substance sulforaphane in flower disc (mg kg^{-1} fresh eight).

Mean B	Sulfur Level			Bio fertilization
	S2	S1	S0	
30.9	38.4	35.1	19.3	B0
38.1	47.8	41.6	24.9	B1
4.33	8.32			LSD
	43.1	38.35	22.1	Mean S
	5.92			LSD

CONCLUSIONS

We conclude from this study that the levels of agricultural sulfur with inoculation with *Thiobacillus* bacteria had a significant role in increasing the growth and yield of broccoli plant, in addition to increasing the concentration of the active substance in it.

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