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Evaluating Different Liquid Organic Fertilizer from Cattle Blood and Mulch Colors for Quality and Quantity Improvement of Garlic

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ABSTRACT

Garlic is widely used as a flavoring due to its sulfur and flavonoid contents. Successful garlic cultivation requires careful soil fertility management and effective weed control strategies. To address these challenges, applying liquid organic fertilizer made from cow's blood and installing mulch are effective solutions. This study was conducted from April to June, 2024 in Temanggung, Indonesia with an altitude of 1100 meter above sea level (masl). The experiment followed a 4×4 factorial design in a split-plot arrangement with three replications. The first factor was the doses of liquid organic cattle blood fertilizer at 0 mL/plant, 6 mL/plant, 12 mL/plant, and 18 mL/plant. The second factor was mulch colors, including silver, black, transparent, and blue mulch. The results showed that higher doses of liquid organic cattle blood fertilizer (up to 18 mL/plant) reduced the sulfur content in garlic bulbs, potentially affecting flavor and storage properties. Furthermore, blue mulch resulted in the highest fresh and dry storage weight per bulb, largest bulb volume and diameter, and greatest yield per square meter. Interaction between cow blood liquid organic fertilizer and mulch color was presented in bulb volume per plant. Therefore, a combination of blue mulch and liquid organic fertilizer from cow blood up to 18 mL/plant can be applied to improve the garlic production, while a further study is needed to assess long-term effects on soil health and garlic quality.

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1. INTRODUCTION

Garlic (Allium sativum L.), a member of the Alliaceae family, is widely cultivated for its culinary and medicinal properties. In general, garlic contains proteins, minerals, vitamins, lipids, amino acids, and secondary metabolites rich in sulfur compounds that provide its distinct flavor, aroma, and pharmacological properties [1]. According to the Agricultural Data and Information Center (2022), the national demand for garlic is 655,355 tons [2]. Despite its high demand, garlic cultivation faces several agronomic challenges, including soil fertility depletion and costly weed management [3]. Furthermore, national production of garlic remains very low, amounting to only 30,194 tons—a 33.02% decrease compared to the 2021 production of 45,092 tons. Consequently, to fulfil the market demand, garlic is heavily imported. Prolonged use of inorganic fertilizers can adversely affect the physical, chemical, and biological properties of soil if not balanced with organic fertilizers. This imbalance can hinder plant growth. Furthermore, the decline in garlic plant production is also influenced by nutrient competition between the main crop and weeds, as well as insufficient sunlight exposure

for the plants. One approach to improving garlic production is the application of fertilizers. The use of organic fertilizers can reduce soil pollution and enhance soil fertility.

Cow blood is a viable raw material for producing liquid organic fertilizer (LOF). Biochemical analysis of cow blood, revealed a total protein content of 6.82 g/L, cholesterol at 166.08 mg/L, glucose at 68.40 mg/L, and calcium at 9.90 mg/L [4]. Identified key chemical elements in cow blood, including Nitrogen (N) 14.9%, Phosphorus (P) 0.45%, Potassium (K) 0.59%, Calcium (Ca) 615 mg/kg, Magnesium (Mg) 405 mg/kg, Iron (Fe) 0.26%, Zinc (Zn) 117 mg/kg, Copper (Cu) 10 mg/kg, Manganese (Mn) 11 mg/kg. Putri (2023), conducted nutrient content tests on cow blood LOF, showing Organic Carbon (C-organic): 4.74%, Nitrogen (N) 2.16%, Phosphorus (P) 0.02%, Potassium (K) 0.04%, Iron (Fe) 156.4 ppm, Manganese (Mn) 4.3 ppm, Zinc (Zn) 3.8 ppm, Boron (B) 37.4 ppm, Cobalt (Co) 0.3 ppm, Molybdenum (Mo) 0.5 ppm. Research by Fidia (2021) demonstrated that applying cow blood LOF at a concentration of 500 mL/L of water yielded the highest results for shallot plants in terms of plant height, tuber weight per plot, and tuber weight per hectare. These results were superior to treatments with concentrations of 0 mL/L, 125 mL/L, 250 mL/L, and 375 mL/L of water. Among various organic fertilizers, cattle blood-derived liquid fertilizer presents a promising solution, repurposing slaughterhouse waste while enriching soil nutrients [5].

Another effective strategy is the use of plastic mulch, which not only suppresses weed growth, but also helps regulate the nutrient and water availability for the plants. The Impact of Silver-Black Mulch and Light Spectrum on Plant Growth. Using silver-black mulch helps maintain consistent soil temperature, which promotes optimal root development and enhances the decomposition of nutrients by microorganisms. Plants with well-developed root systems can absorb water and nutrients more efficiently, leading to more effective photosynthesis. The glucose compounds produced during photosynthesis are then used for leaf formation [6]. Different colors of mulch have varying effects on the reflected light spectrum. The light spectrum, from longest to shortest wavelength, includes Red: 625-740 nm, Orange: 590-625 nm, Yellow: 565-590 nm, Green: 520-565 nm, Blue: 435-520 nm, Violet: 380-435 nm [7].

White sunlight, when refracted or dispersed, breaks down into red, orange, yellow, green, blue, and violet. This spectrum is crucial for plants to perform photosynthesis [8]. Emphasize that light color significantly influences plant growth, and not all light colors have wavelengths compatible with chlorophyll in plants. Blue and red light are particularly beneficial for increasing leaf width and stem height because chlorophyll absorbs blue light efficiently, optimizing the photosynthetic process. Several plastic mulch colors that are commonly used in garlic cultivation are silver, black, transparent, and blue [9]. Therefore, this study investigated the optimal application rate of cattle blood-derived liquid fertilizer and evaluates the impact of different mulch colors on garlic bulb yield and quality.

2. METHODS

This study was conducted from March to June 2024 in Gembyang Hamlet, Kentengsari Village, Temanggung Regency, Central Java, Indonesia (1,100 masl). The tools used in this study were: Hoes, rakes, measuring tapes, raffia ropes, knives, stakes, scales, meters, planting tools, buckets, mulch punchers, writing tools, cameras, name labels, and sprayers. Meanwhile, the materials used in this study were: Lumbu Hijau garlic seeds, cow blood liquid organic fertilizer (POC), silver mulch, black mulch, transparent mulch, blue mulch, base fertilizers, insecticides, and fungicides. This study employed a 4×4 factorial experiment arranged in a split-plot design with three replications (blocks). The first factor was the dosage of liquid organic fertilizer derived from cattle blood (POC), with four levels: D0 = 0 mL/plant, D1 = 6 mL/plant, D2 = 12 mL/plant, and D3 = 18 mL/plant. The second factor was the mulch color, consisting of M1 = silver, M2 = black, M3 = transparent, and M4 = blue. Liquid fertilizer applications often require methods that cover larger areas, such as sprinkling or irrigation. Liquid fertilizer doses as main plots can apply this treatment to larger and more uniform areas, minimizing application errors and variability between plots, while mulch color is easier to apply and change in smaller areas, making it relatively easy to install mulch with different colors in small sections of each main plot.

Using different dosages of Liquid Organic Fertilizer (LOF) and mulch colors to determine the optimal dosage and mulch color that can enhance garlic biomass formation. The study stages began with land and materials preparation, mulch installation, planting, maintenance (including watering, weeding, replanting, earthing up, pest and disease control), and harvesting. The observed parameters included

1. Fresh Bulb Weight : The fresh weight of the bulbs is measured after harvesting. First, the bulbs are cleaned of any dirt and separated from the foliage. They are then weighed using an analytical balance.
2. Dry Storage Weight : Dry storage weight is determined by first measuring the fresh bulb weight. The bulbs

are then air-dried under direct sunlight until their moisture content reaches approximately 43%. Finally, they are weighed using an analytical balance.

3. Bulb Volume : Bulb volume is obtained by subtracting the initial water volume from the final water volume in a measuring cylinder after the bulbs have been submerged.
4. Fresh and Dry Bulb Weight per Square Meter (m^2) : The fresh and dry bulb weight per m^2 is calculated by measuring the bulb weight within a single plot area and then converting it to a per m^2 basis.
5. Bulb Diameter : Bulb diameter is measured by horizontally slicing the bulb and then using a ruler to take the measurement.
6. Flavonoid Content Testing : For flavonoid content testing, 10 g of garlic bulb is crushed using a mortar and dissolved in 10 mL of distilled water to obtain an extract. The garlic extract is then mixed with 96% alcohol in a 2:3 ratio (2 mL garlic extract: 3 mL 96% alcohol). This mixture is shaken until homogeneous and filtered. From the filtrate, 0.5 mL is taken and 1.5 mL of ethanol, 0.1 mL of AlCl3 (Aluminum chloride), 0.1 mL of 1 M CH3COOK (Potassium acetate), and 2.8 mL of distilled water are added. The mixture is then incubated for 30 minutes at 25°C. Testing is performed using a UV-Vis Spectrophotometer at a wavelength of 349 nm.
7. The sulfur content test was conducted using 96% ethanol as a solvent. First, 100 grams of peeled garlic were weighed and then crushed in a porcelain mortar. The crushed garlic was transferred to a beaker, and 20 mL of ethanol were added and stirred. The beaker was then covered and wrapped with plastic, after which it was left to stand for 24 hours to form an ethanol-garlic mixture. Finally, 10 mL of the extracted mixture were taken for GC-MS analysis.

All data were analyzed using analysis of variance (ANOVA). If significant differences were found, further testing was performed using orthogonal polynomial tests for the liquid organic fertilizer dose treatment factor and the least significant difference (LSD) test with confidence levels of 5% and 1%.

3. RESULTS AND DISCUSSION

The results were analyzed using analysis of variance (ANOVA), which produced F-values indicating the effects of cattle blood-based liquid organic fertilizer dosage, mulch color, and their interaction, as presented in Table 1.

Table 1. F-Test Values

Observation parameters	F-test value		
	Dosage	Mulch	Interaction
Fresh bulb weight per plant	2.034 ^{ns}	27.276**	1.099^{ns}
Dry storage bulb weight per plant	0.746 ^{ns}	13.637**	1.542^{ns}
Bulb volume	1.264 ^{ns}	45.827 **	2.453*
Fresh bulb weight per m^2	1.753 ^{ns}	29.549**	1.427^{ns}
Dry storage weight per m^2	1.762 ^{ns}	32.989 **	0.827^{ns}
Bulb diameter	3.636^{ns}	15.082**	0.736^{ns}
Sulfur content	16.259**	0.915^{ns}	0.662^{ns}
Flavonoid content	0.760^{ns}	4.307**	1.330^{ns}

Note: * = significantly different; ** = highly significant different; ns = not significantly different

The dosage of cattle blood-based liquid organic fertilizer only had a significant effect on sulfur content, while different colored-mulch showed a *vice-versa* condition. Interactions between POC dosage and mulch color were significantly different only on bulb volume per plant.

3.1. Effects of Cattle Blood-Based Liquid Organic Fertilizer Dosage on the Sulfur Content of Garlic Bulbs

As cattle blood-based liquid organic fertilizer dosage had a significant effect on sulfur content in garlic bulbs (Table 1), a further analysis using an orthogonal polynomial test was conducted and presented in Fig. 1. Increasing dosage of POC presents a declined sulfur content of the garlic bulbs. This phenomenon is attributed to the presence of iron (Fe) in the cattle blood-based liquid organic fertilizer, which induces chemical interactions affecting nutrient absorption by the plant [10]. Sulfur and iron are essential elements that interact within the plant root absorption system. Their biochemical interactions affect sulfur uptake. Excessive iron can disrupt plant metabolism, including pathways involved in the production of sulfur- containing compounds. At

high concentrations, excess iron interferes with the synthesis of organosulfur compounds—key constituents of garlic—ultimately reducing sulfur content in the bulbs [11].

In the soil, iron primarily exists in the form of Fe^{2+} or Fe^{3+} ions, which can interact with sulfur to form complexes that reduce sulfur availability to plants. These complexes bind sulfur, making it less accessible for plant uptake and leading to lower sulfur levels in garlic bulbs [12].

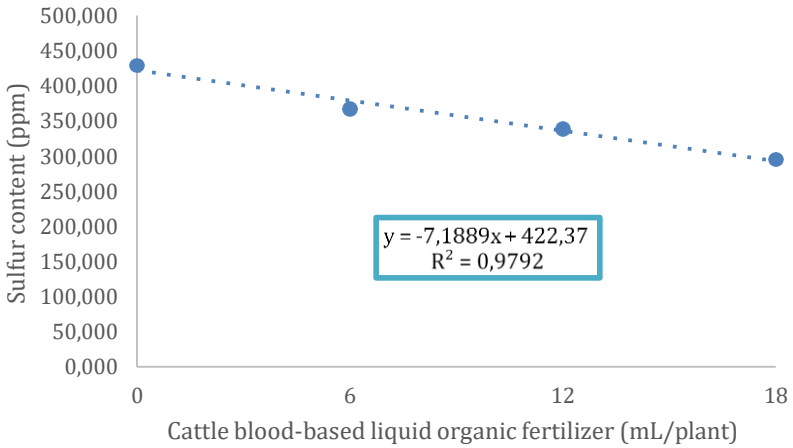


Fig. 1. The Effect of Cattle blood-based liquid organic fertilizer Dosage on Sulfur Content of Garlic

3.2. Effects of Mulch Color on the Quality and Quantity of Garlic Bulbs

As mulch colors had a highly significant effect on fresh bulb weight per plant, dry storage bulb weight per plant, bulb volume per plant, fresh bulb weight per square meter, dry storage weight per square meter, bulb diameter, and flavonoid content (Table 1), a further analysis was employed on each parameter and presented in Table 2.

Table 2. Post Hoc Test Results for the Effect of Mulch on Parameters

Parameter	Silver Mulch (M1)	Black Mulch (M2)	Transparent Mulch (M3)	Blue Mulch (M4)	LSD 1 %
Fresh bulb weight per plant	8.861[a]	5.417[b]	4.722[b]	10.139[a]	1.759
Dry storage bulb weight per plant	6.924[a]	3.847[b]	3.319[b]	7.556[a]	1.331
Bulb volume	7.889[b]	4.444[c]	3.972[c]	9.431[a]	1.340
Fresh bulb weight per m²	20.999[b]	19.453[b]	16.713[c]	23.446[a]	2.053
Dry storage weight per m²	18.702[b]	17.072[b]	15.195[c]	21.032[a]	1.706
Bulb diameter	5.321[b]	4.859[b]	4.739[b]	5.592[a]	0.409
Flavonoid content (ppm)	109.292[c]	122.083[b]	128.042[ab]	134.250[a]	11.845

Note: Values followed by the same letter are not significantly different according to the LSD test at 1%.

Table 2 presents the LSD results at a 1% significance level evaluating the effects of various types of mulch on onion growth and quality parameters. Blue mulch (M4) consistently showed the most outstanding performance among all types of mulch tested. Specifically, blue mulch produced the highest fresh bulb weight per plant (10,139) and the highest dry bulb weight per plant (7,556), both of which were significantly higher than the other mulches. Similarly, bulb volume and dry bulb weight per \text{m}^2 also reached the highest values with blue mulch, at 9,431 and 21,032, respectively. Flavonoid concentration, an important indicator of bulb quality, was also highest in the blue mulch treatment (134,250 ppm), suggesting potential for enhanced nutritional value. The dominance of blue mulch indicates its ability to create an optimal microenvironment for onion bulb growth, possibly through beneficial modifications to light spectrum or soil temperature.

Blue mulch (M4) indicates the highest values for fresh bulb weight per plant, dry storage bulb weight per plant, bulb volume per plant, fresh bulb weight per square meter, dry storage weight per square meter, bulb diameter, and flavonoid content. This condition is attributed to the role of blue mulch in optimizing sunlight radiation, stabilizing soil temperature and moisture, and preventing drought, thereby supporting optimal plant

growth. Additionally, blue mulch also helps maintain nutrient balance, which enhances plant growth and optimizes nutrient absorption for photosynthesis and bulb formation [13].

Blue mulch reflects sunlight, maintaining temperature and facilitating the accumulation of photosynthates in bulbs [13]. That blue mulch can reflect light and lower soil temperatures, maximizing sunlight absorption, which optimizes photosynthesis product, including fresh and dry bulb weights. The highest bulb volume was also observed with blue mulch, attributed to its ability to create an ideal microenvironment and nutrient conditions, regulating the root growth improvement [14]. Light directly impacts plant metabolism during photosynthesis and indirectly affects plant growth and development [15]. Blue plastic mulch effectively transmits and reflects light, helping to regulate plant temperature and optimize conditions for photosynthesis and garlic bulb formation. In addition, mulch modifies the microclimate by increasing soil temperature during colder seasons and reducing heat stress in warmer periods, ensuring environmental suitability for plant growth. Blue mulch reflects a greater amount of radiation back into the atmosphere, contributing to soil temperature and moisture stability [16].

Flavonoids, a group of secondary metabolites found in plant tissues, are influenced by environmental conditions [17]. That plants respond to elevated temperatures by synthesizing antioxidant compounds as a protective mechanism. Blue and silver mulch enhance light reflection back to the canopy, improving sunlight absorption and increasing photosynthetic efficiency. This process leads to greater production of photosynthates, which are subsequently stored in bulbs [18]. As a result, both blue and silver mulch yielded comparable fresh bulb weights per plant.

Although blue mulch showed superiority, silver mulch (M1) also performed well for some parameters, such as fresh bulb weight per plant (8,861) and dry bulb weight per plant (6,924), which were significantly higher than black and transparent mulch. However, for bulb volume and dry bulb weight per \text{m}^2, silver mulch tends to be lower than blue mulch. Black mulch (M2) and transparent mulch (M3) generally showed lower performance compared to blue and silver mulch for most bulb growth parameters, including bulb volume, fresh and dry bulb weight per plant, and per \text{m}^2. Nevertheless, transparent mulch exhibits higher flavonoid content (128,042 ppm) compared to silver and black mulch, though still lower than blue mulch. This pattern highlights that the selection of mulch type has a significant and varied impact on various aspects of plant growth and quality, with blue mulch emerging as the most effective choice for enhancing tuber productivity and quality based on the presented data.

Transparent mulch (M3) yielded the lowest values for fresh bulb weight per plant, dry storage bulb weight per plant, bulb volume per plant, fresh bulb weight per square meter, dry storage weight per square meter, and bulb diameter. This effect is attributed to the high transmissivity of transparent mulch, which allows direct sunlight penetration into the soil, leading to excessive soil temperatures. Elevated soil temperatures accelerate respiration, diverting photosynthates from storage to energy metabolism, thereby reducing photosynthetic efficiency for bulb enlargement [19]. Additionally, transparent mulch facilitates weed growth, leading to nutrient competition with garlic plants and ultimately reducing garlic yields.

3.3. Interaction Effects of Cattle Blood-Based Liquid Organic Fertilizer and Mulch Color

As a significant interaction is present between the dosage of cattle blood-based liquid organic fertilizer and mulch color on bulb volume (Table 1), the interaction effect was further analyzed using orthogonal polynomial tests, as shown in Fig. 2. The orthogonal polynomial tests analysis results indicate an equation for the interaction of cattle blood-based liquid organic fertilizer and transparent mulch (M3): $y= -0.001x^2 + 0.0291x + 2.2937$. This means that black mulch responded optimally to a POC dosage of 15 mL/plant, resulting in the highest bulb volume of 3.722 mL

The results indicate variations in bulb volume across different dosages of liquid organic fertilizer derived from cow blood (POC), as the supplied nutrients were assimilated through photosynthesis, facilitating plant growth and photosynthate accumulation. Bulb size is significantly influenced by nutrient availability, particularly nitrogen, which plays a crucial role in vegetative growth and the storage of photosynthates as energy reserves.

Microelements in cow blood POC, such as iron (Fe), play a vital role in chlorophyll synthesis and electron transport chain enzymes. Deficient Fe impairs chlorophyll biosynthesis, disrupts protein assembly, reduces ribosome abundance, decreases pigment concentration, and diminishes enzyme activity, ultimately affecting overall plant metabolism and growth [20].

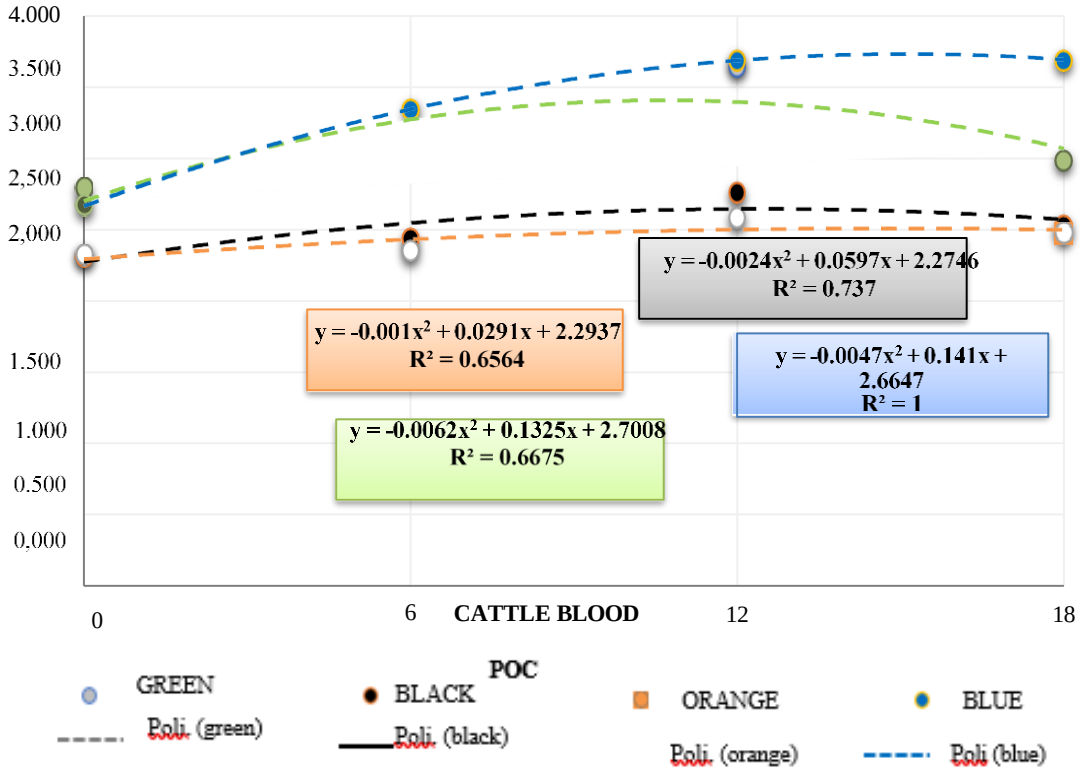


Fig. 2. The interaction of cattle blood-based liquid organic fertilizer and mulch color on bulb volume

Weeds negatively affect garlic productivity by competing for nutrients that should be utilized by the crop. Transparent mulch (M3) obtained the lowest bulb volume due to high soil moisture, which induced weed growth, leading to nutrient competition. In contrast, blue mulch creates an ideal microenvironment with optimal nutrient availability to assist root development. Moreover, black mulch increased soil temperature, potentially disrupting the decomposition of nutrients. Therefore, the results suggest that environmental conditions created by different mulch colorations can significantly influence the beneficial effect of cattle blood-based liquid organic fertilizer in promoting optimal bulb volume.

4. CONCLUSION

Increasing the dosage of cattle blood-based liquid organic fertilizer (POC) to 18 mL/plant reduced the sulfur content in garlic bulbs from 429.333 ppm to 295.167 ppm, which was attributed to the heavy metal content sin the POC, which react with sulfur in the soil to form insoluble sulfide compounds. Iron primarily exists in the form of Fe^{2+} or Fe^{3+} ions, which can interact with sulfur to form complexes that reduce sulfur availability to plants. The application of blue mulch presents the highest fresh bulb weight per plant, dry bulb weight per plant, bulb volume, fresh bulb weight per square meter, and flavonoid content. Black mulch responded optimally to POC at a dosage of 15.370 mL/plant, yielding an optimal bulb volume of 9.307 mL. This trend continued at 18 mL/plant, further lowering sulfur levels. Volatile sulfur compounds are responsible for the pungent, sharp taste and strong, characteristic aroma of onions. A decrease in the concentration of these volatile sulfur compounds will lead to a reduction in both the taste and smell.

Author Contribution

All authors contributed equally to the main contributor to this paper. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest
The authors declare no conflict of interest.

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