

EFFECT OF EM4 (EFFECTIVE MICROORGANISM 4) ON GROWTH AND PRODUCTIVITY OF CUCUMBER (Cucumis sativus L.)

PENGARUH EM4 (EFFECTIVE MICROORGANISM 4) TERHADAP PERTUMBUHAN DAN PRODUKTIVITAS KETIMUN (*Cucumis sativus* L.)

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Submitted: 5 February 2024; Revised: 24 June 2024; Accepted: 14 August 2024

Abstract

Cucumber (*Cucumis sativus* L.) is a nutritious and healthy vegetable that is commonly consumed by Indonesian people. To fulfill self-sufficiency for household scale needs during the COVID-19 pandemic, cucumber cultivation can be carried out in home gardens, using containers such as polybags. Growing cucumbers on limited land requires a carefully optimized planting media composition by applying Effective Microorganism 4 (EM4) to the polybag media when planting. The research has been conducted which aims to determine the best EM4 dosage for the growth and productivity of cucumbers. The study used a Randomized Block Design consisting of control and three treatment doses of 10% concentration EM4, namely 20, 40, and 60 mL per polybag with six replications. The planting media used is a mixture of loam soil and goat manure. NPK fertilizer is given as an additional nutrient. The EM4 application is done by pouring it every eight days into the planting media in polybags. The results showed an increase in growth parameters and productivity of cucumber plants namely plant height, leaf chlorophyll content, time of flower emergence, number of flowers, and number of flowers that form fruit. 40 mL EM4 is the dose that showed the highest growth and productivity.

Keywords: Cucumber; Cucumis sativus; Effective Microorganism 4

Abstrak

Mentimun (Cucumis sativus L.) merupakan sayuran bergizi dan menyehatkan yang banyak dikonsumsi masyarakat Indonesia. Untuk memenuhi swasembada kebutuhan skala rumah tangga di masa pandemi COVID-19, budidaya mentimun dapat dilakukan di pekarangan rumah, dengan menggunakan wadah polybag. Menanam mentimun di lahan terbatas memerlukan optimalisasi komposisi media tanam secara cermat dengan menerapkan Effective Microorganism 4 (EM4) pada media dalam polybag. Telah dilakukan penelitian yang bertujuan untuk mengetahui dosis EM4 terbaik untuk pertumbuhan dan produktivitas tanaman mentimun. Penelitian menggunakan Rancangan Acak Kelompok yang terdiri atas kontrol dan tiga perlakuan dosis EM4 konsentrasi 10% yaitu 20, 40, dan 60 mL per polybag dengan enam ulangan. Media tanam yang digunakan adalah campuran tanah lempung dan kotoran kambing. Pupuk NPK diberikan sebagai unsur hara tambahan. Penerapan EM4 dilakukan dengan cara disiram setiap delapan hari sekali ke dalam media tanam di polybag. Hasil penelitian menunjukkan adanya peningkatan parameter pertumbuhan dan produktivitas tanaman mentimun yaitu tinggi tanaman, kandungan klorofil daun, waktu munculnya bunga, jumlah bunga, dan jumlah bunga yang membentuk buah. Dosis yang menunjukkan pertumbuhan dan produktivitas tertinggi adalah 40 mL EM4.

Kata Kunci: Cucumis sativus; Effective Microorganism 4; Mentimun

Permalink/DOI: http://dx.doi.org/10.15408/kauniyah.v18i1.37561

INTRODUCTION

Cucumber (*Cucumis sativus* L.) which belongs to the *Cucurbitaceae* family is one of the annual cultivated plants consumed by the Indonesian people. Cucumber contains minerals including N, P, K, Ca, Mg, Na, Cu, Zn, Fe, Mn, and B (Jang et al., 2021). Every 100 g of cucumber fruit contains 2 mg Na, 147 mg K, 24 mg P, 16 mg Ca, and 13 mg Mg (Changade & Ulemale, 2015). Cucumber fruit contains secondary metabolites including glycosides, saponins, beta carotene, alpha carotene, chlorophyll a, chlorophyll b, tannins, flavonoids (anthocyanins), alkaloids, resins, folate, steroids, cholesterol, cryptoxanthin B, lutein zeasantin, γ -tocopherol, alpha-tocopherol, choline, and betaine (Uthpala et al., 2020). In addition, cucumbers also contain vitamins A, C, E, and K, niacin (vitamin B13), thiamin (vitamin B1), riboflavin (vitamin B2), pyridoxine, and pantothenic acid. Every 100 g of cucumber contains 2.8 mg of vitamin C, 0.03 mg of vitamin E, and 16.4 µg of vitamin K (Changade & Ulemale, 2015). With its various ingredients, cucumber fruit is useful as an antioxidant, diuretic, hypolipidemic, anti-inflammatory, and antihyperglycemic. Besides being rich in nutrients, cucumbers also have a fresh and cooling taste for consumption (Uthpala et al., 2020).

During the COVID-19 pandemic, cucumber consumption on a household scale can be fulfilled by cultivating in the land around the house as long as it meets the growth requirements. Cucumber needs fertile-loose soil, rich in humus, not waterlogged, and has a pH ranging from 6–7. Cucumber requires a growing medium consisting of primary macronutrients (N, P, K), secondary macronutrients (Ca, Mg, and S), and micronutrients (B, Fe, Mn, Cu, Zn, and Mo) (Singh, 2017). Nutrients in growing media are obtained from organic matter that affects aeration, structure, drainage, humidity, soil field capacity, and soil microbial ecology (Bonilla et al., 2012). In addition to the presence of nutrients, the quality of good growing media is also influenced by the availability of stable aggregates, able to be penetrated by roots, with low levels of weeds and diseases, and supported by good biological activity (Seifu & Elias, 2018). Biological activity in growing media can be increased by adding Effective Microorganisms (EM), an inoculant containing selected microorganisms that can increase microbial populations in soil and water (Abdulkareem et al. 2020).

Applying EM to soil can improve soil quality and fertility, due to its ability to decompose and ferment the organic fractions in the soil from plant residues and convert them into humus which contains nutrients that can enhance plant growth. The first beneficial solution of microorganisms contained more than 80 microbial species from 10 genera, isolated in Japan. However, with time, the technology was refined to include predominant populations of lactic acid bacteria and yeast and smaller numbers of photosynthetic bacteria, actinomycetes, and other types of microorganisms. All of these are compatible with each other and can coexist in liquid culture (Higa & Parr, 1994). Microorganisms included in EM are lactic acid bacteria (e.g., Lactobacillus plantarum, L. casei, and Streptococcus lactis), photosynthetic bacteria (e.g., Rhodopseudomonas palustris), yeast (e.g., Saccharomyces spp.), and fungi. Condor Golec et al. (2007) described these effective microorganisms as follows, photosynthetic bacteria (phototrophic bacteria) are independent selfsupporting microorganisms. Those bacteria synthesize amino acids, nucleic acid, bioactive compounds, and sugar using exudate from roots, organic matter, sunlight, and geothermal from the ground as an energy source. The resulting metabolites can be absorbed by plants or as a substrate for other bacteria, thereby increasing the biodiversity of soil microflora. In addition, EM can improve the composition of soil particles, so that it can retain nutrients and moisture. Thus, EM can increase the availability of nutrients in the soil for plants, thereby reducing the constant use of fertilizers and planting costs (Joshi et al., 2019)

The efficacy of using EM depends on the ratio of dominant microorganisms in the culture mixture. EM are divided into four types containing mixed cultures of various species of microorganisms with different percentages, namely EM1, EM2, EM3, and EM4. EM1 is a mixed culture that predominantly contains filamentous fungi which are hot resistant and accelerate the decomposition of organic amendments. Originally developed to prepare compost quickly but is no longer being produced. EM2 is used primarily to protect crops from soil-borne pathogens, diseases, and insects. Mainly contains species of the genus *Streptomyces* which produces antibiotics that suppress harmful microorganisms. It also contains small numbers of photosynthetic bacteria, yeast,

and fungi. EM3 is a mixed culture that is relatively dominant in photosynthetic bacteria with a smaller number of yeast and actinomycetes (Javaid, 2010). EM4 is a mixed culture of mostly *Lactobacillus* and a few other microorganisms. The type commonly used in Indonesia is EM4. Effective Microorganisms 4 (EM4) contains lactic acid bacteria (*Lactobacillus* sp.), photosynthetic bacteria (*Rhodopseudomonas* sp.), *Actinomycetes* sp., *Streptomyces* sp., yeast, and cellulose-degrading fungi (Diver, 2001). Furthermore, EM4 also contains *Saccharomyces* sp. as well as other functional bacteria (nitrogen fixer, phosphate solubilizing bacteria, hormone-producing bacteria, and decomposers). Due to the content of lactic acid bacteria and photosynthetic bacteria, EM4 helps decompose and ferment the organic fractions in the soil decompose plant residues, and convert them into nutrient-rich humus (Abdulkareem et al., 2020).

The application of EM has been carried out in the cultivation of several plants, such as green beans (Javaid & Bajwa, 2011), chilies (Syafruddin & Safrizal, 2013), pumpkins (Olle & Williams, 2015), tomatoes (El Kiyumi et al., 2017), peanuts (Iriti et al., 2019), and cucumber (Olle & Williams, 2015). The results of all these studies show that the application of EM to the planting medium during the pre-treatment period (before planting) and during the planting period causes an increase in plant growth. Olle and Williams (2015) reported the application of 8% EM4 to cucumber plants only until the growth of the vegetative phase and resulted in lower growth than controls, but it was reported that EM4 could increase plant growth during flowering (generative phase). This was also proven in the research of Syafruddin and Safrizal (2013) who used chili plants. Joshi et al. (2019), and Olle and Williams (2015) reported that an optimum EM dose range is 32–64 mL/polybag. Based on this, a study was conducted to determine the best dose of EM4 among 0 (control), 20, 40, and 60 mL for the growth and productivity of cucumber (*Cucumis sativus* L.) in polybag container. The growth parameters measured consisted of plant height, chlorophyll content, days to emergence of flowers, number of flowers, and percentage of flowers that set and develop into fruit.

MATERIALS AND METHODS

Research Time and Place

A polybag experiment was conducted in the front yard of the house in Jurangmangu Barat area, Pondok Aren, South Tangerang, Indonesia. The research location is at an altitude of about 25 meters above sea level, from March to June 2022.

Research Materials and Tools

The materials used in this study were *Cucumis sativus* seeds of Swiss 1 (Unggul Jawara), goat manure, EM4 (PT. Songgolangit Persada), NPK Mutiara fertilizer (16:16:16), sodium hypochlorite (Bayclin), 80% acetone, insecticide (Decis 25 EC). The equipment used for leaf chlorophyll content is a UV-Vis spectrophotometer, centrifuge, Soil Tester 4 in 1 LCD Digital pH Meter Soil Tester Analyzer Hygrometer. The tools used in this research are polybags (15×15 cm), polybag (40×40 cm), length measuring instrument, and weight measuring instrument,

Research Design

The study used a Randomized Block Design with control (0) and three treatments, namely 20, 40, and 60 mL of 10% EM4 solution. Each treatment was replicated 3 times. One individual plant in one polybag is considered as one replication.

Seed Germination and Transplantation

Cucumber seeds are soaked in water to select viable seeds. The viable seeds were then surface sterilized with 1% sodium hypochlorite for 3 min followed by several washings with sterilized water. The seeds were then placed on wet filter paper in a closed container and allowed to stand for 12 hours. Germinated seeds are planted on media consisting of rice husk. The three-week-old seedlings (having 3 leaves) were transplanted into polybags (40×40 cm) filled with ± 5 kg mixture of loam soil and goat manure. Each polybag contains only one plant.

EM4 Treatment

The EM4 treatment was started one week before the transplanting of the plants from the germination medium with an interval of every 8 days until the end of the experiment (42 days of observations, in total). The application of 10% EM4 solution was poured at the edge of the polybag. During the six-week experiment, ambient temperature and humidity were measured twice a day (at 1 and 8 pm). The pH level of the media was measured every week. Soil pH was measured using a Soil Tester 4 in 1 LCD Digital pH Meter Soil Tester Analyzer Hygrometer. The pH sensor measurement tool is inserted to a depth of 5 cm from the media surface. The rainfall frequency and duration are also recorded.

Plant Maintenance

Plant maintenance is carried out by watering with tap water 1-2 times per day according to field capacity. But if it rains, the frequency of watering is reduced because enough water is available to plants. NPK fertilizers (16:16:16) were applied in the experiment as much as 100 kg/ha or equal to 1.6 g/polybag dissolved in 160 mL of water repeated every 10 days (Karamina et al., 2020). Pest control using the insecticide Decis 25 EC at a dose of 28 mL/14 L for 1 m² of soil (Lestari & Andrian, 2017). Removal of weed is done mechanically by hand.

Harvesting and Growth Parameter Measurement

The observed vegetative growth parameter was plant height, which was measured every week for six weeks. The generative growth parameters recorded were the days of flower emergence, the number of flowers, and the percentage of fruit set (i.e. the percent ratio between the number of flowers and fruits). Observation is done every two days. The criteria for flowers that are counted are flowers that already fully bloomed, while flowers that form fruit are those that are ± 5 cm in size.

Leaf Chlorophyll Content

Leaf chlorophyll content was measured by UV-Vis spectrophotometer according to Zhao et al. (2019a). Leaf samples were taken from three different individuals at the same position, namely the seventh leaf position from the bottom of each treatment plant. The leaves are washed under running water and dried. Leaf veins were removed while the leaf blade was cut into smaller pieces. 30 mg of leaves were ground using a mortar and pestle. The fine leaf tissue was then added with 10 mL of 80% acetone. The leaf extract solution was pipetted into a Falcon tube and then centrifuged at 3000 rpm for 15 minutes. The resulting supernatant was pipetted into a cuvette, then the absorbance was measured at 663 nm and 645 nm. The absorbance data is then calculated using the formula according to Arnon (1949) as follows. Chlorophyll a (mg/L)= $(12,7 \times A_{663}) - (2,69 \times A_{645})$; chlorophyll b (mg/L)= $(22,9 \times A_{645}) - (4,68 \times A_{663})$; and total chlorophyll (mg/L)= $(20,2 \times A_{645}) + (8,02 \times A_{663})$.

Data Processing and Statistical Analysis

Vegetative and generative growth parameter data; plant height, leaf chlorophyll content, days to emergence of flowers, number of flowers, and the percentage of fruit set were analyzed using the IBM SPSS Statistics version 25. All data obtained was first tested for homogeneity and normality as a condition for the ANOVA test. If the data are normally distributed and homogeneous, then proceed with the ANOVA test. The ANOVA test used in this study is a one-way ANOVA because it only divides the data into groups based on one factor, namely the EM4 dose. The test was continued if there were significant differences between the EM4 dose treatments using the Least Significant Difference (LSD) test to see where the differences were between groups. The LSD method aims to calculate the smallest significant difference by comparing the two mean values (Ostertagová & Ostertag, 2013).

RESULTS

Plant Height

Analysis of variance showed that plant height was significantly different at $P \le 0.05$ between control and EM4 dose treatment. The results of the LSD test showed a significant difference at P

 \leq 0,05 in plant height between the control and all the plant EM4-treated. Increasing the dose of EM4 appeared to increase plant height (Figure 1).



Figure 1. Effect of EM4 application on increase of cucumber plant height. Vertical bars show the standard error of means of 6 replicates

Days to Flower Emergence

Analysis of variance showed significant differences at $P \le 0.05$ on the days to flower between the EM4 treatment compared to control. This significant difference also occurred in the earlier emergence of flowers in the EM4 treatment compared to the control. The EM4 has an important influence on the timing of flowering. In all treatments, flowering took place between 27 and 33 Days After Planting (DAP). Plants with EM4 doses of 40; 20; and 60 mL produced flowers at 27; 28; and 29 DAP respectively, earlier than control plants (33 DAP) (Figure 2).



Figure 2. Effect of EM4 application on days to flowering of cucumber expressed in Days After Planting (DAP). Vertical bars show the standard error of means of 6 replicates



Figure 3. Effect of EM4 on several cucumber flowers and fruits. Vertical bars show the standard error of means of 6 replicates

The results of the LSD test showed that there was a significant difference at $P \le 0.05$ between the control and EM4 treatment on the number of flowers at a dose of 20; 40; and 60 mL (Figure 3). The effect of EM4 was significant for chlorophyll a and total chlorophyll content but was insignificant for chlorophyll b as shown by analysis of variance. However, the average contents of chlorophyll a, b, and total leaves of all plants treated with EM4 were higher than control (Figure 4).



Figure 4. The effect of EM4 dose on chlorophyll content of cucumber leaves. The bars represent the standard error

The LSD test showed that there was *a* significant difference at $P \le 0.05$ of a, b, and total chlorophyll between the plant leaves control and the 40 mL EM4-treated. There was a positive effect of the use of EM4 on chlorophyll levels. The value of chlorophyll content (Figure 4) is also directly proportional to the number of flowers and the number of flowers that form fruit at the dose of EM4 40 mL> 20mL> 60mL.

Environmental Data

Soil pH measurements were carried out during the six weeks of the growing season. The average weekly soil pH value for all treatments was 7. This indicated that the soil pH had not changed in the presence of EM4 and was still within the range of pH which is good for cucumber plant growth.

DISCUSSION

Our results showed that increasing the dose of EM4 increased plant height; this result is also supported by previous findings from Chantal et al. (2013) who reported that bioorganic fertilizer with EM significantly increased the height of tobacco plants. The significantly different increase in plant height due to being given EM4 shows the role and function of EM4 (Joshi et al., 2019). Effective microorganisms can increase plant growth by improving soil quality and the diversity of microorganisms (Joshi et al., 2019). The beneficial microorganisms in EM4 applied together with goat manure and NPK caused a significant increase in cucumber plant nutrient uptake at the final stage of cucumber plant growth. Improving soil quality is usually characterized by increasing soil cation exchange capacity (CEC) (Spargo et al., 2013), aeration, and organic matter content (Higa & Parr, 1994). This is facilitated by EM4 which can increase the release of nutrients from organic materials such as goat manure, with rapid decomposition into carbon dioxide and mineral elements (Kumari & Chaudhary, 2020). This causes an increase in ion binding sites on soil particles (Spargo et al., 2013). Apart from that, yeast as a constituent of EM4 can produce bioactive substances such as phytohormones and enzymes, so it is likely that it can trigger vegetative growth as indicated by plant height. These results are also supported by the findings of Khaliq et al. (2006) who reported that the application of EM combined with organic materials and NPK fertilizer increased cotton yields significantly compared to mixed media amendments without the application of EM.

Besides vegetative growth, the EM4 has an important influence on the timing of flowering. In all treatments flowering took place earlier than in control plants. Flower initiation and development are affected by many factors, such as plant age, temperature, photoperiod, and plant hormones. In addition, plant nutrient status can also affect flower initiation and development. The induction of flowering is related to the ratio of carbohydrates and nitrogen (C: N) in plants. A high C/N ratio is considered to promote reproductive growth, while a low C/N ratio is associated with vegetative growth or even inhibits flowering. Photosynthesis plays an important role in flowering induction, differentiation, and flower initiation. Zhao et al. (2019b) noted that the application of beneficial microorganisms significantly increased the availability of nutrients, organic matter, and total nitrogen in the soil.

The productivity of cucumber plants was also influenced by the administration of EM4, this could be seen in the percentage of flowers that formed fruit between control plants and those given a dose of 40 mL, which was 45%, plants with a dose of 60 mL was 42%, while the lowest percentage was given a dose of 20 mL. Flower and fruit development is influenced by the availability of phosphorus in the planting substrate. This is because P plays a role in nucleic acid synthesis, membrane stability, and the availability of energy (ATP) which is used for the initiation of flowering and fruit development (Sinha & Tandon, 2020). The goat manure used as a media mixture contains phosphorus and potassium. However, the problem that often occurs is that P is an element that has very low availability in the soil because its diffusion rate is very low and it is more often bound to other compounds so that it cannot be absorbed by plants (Shen et al., 2011). In this case, EM4 decomposes or ferments organic matter into a form that is available to plants during the flowering and generative stages (Joshi et al., 2019).

The average number of flowers and the number of flowers that form fruit are proportional to the EM4 dose given 40>20>60 mL. This is because the leaves taken for measuring chlorophyll levels are leaves in the flowering phase chlorophyll can act as a visible-light photoredox catalyst in the process of photosynthesis (Hu et al., 2018). The photosynthetic capacity of a leaf is affected by the amount of chlorophyll content (a, b, and total chlorophyll).

These results were also proportional to the time the flowers appeared, namely the 40 mL treatment flowered faster than 20 mL, and the 20 mL treatment was faster than 60 mL (Figure 2). This is thought to be due to the maximum field capacity of polybags when given 40 mL of EM4 solution so that when given 60 mL decreased productivity. When the soil in the polybag has reached its maximum field capacity, water and other elements can flow out of the polybag (Rai et al., 2017). This also occurs in phosphorus which is needed for initiation of flowering and fruit development. Phosphorus in the form of phosphate obtained through EM4 mineralization is an element that has a very low diffusion rate and a negative charge (anion) that is the same as the soil charge (Sinha & Tandon, 2020; Uchida, 2000; Johan et al., 2021). The same charge between phosphorus and soil makes the soil unable to bind forms of phosphorus that can be absorbed by plants (Johan et al., 2021). Thus, when the planting medium in polybags has reached maximum field capacity, the availability of phosphorus for plants will decrease because the phosphorus in the planting medium is leached and lost along with excess water.

Another parameter that also determines plant yield is chlorophyll, especially the nitrogen content of the planting medium. Increasing the availability of nitrogen in the soil can increase the chlorophyll content of plants, which ultimately increases the rate of plant photosynthesis. Sugar as photosynthate will be allocated for flower and fruit development. Thus, the chlorophyll content of leaves in the flowering phase will be directly proportional to plant productivity which is manifested in the number of flowers and flowers that form fruit. EM4 can increase the decomposition of soil organic matter thereby producing nutrients that can be absorbed by plants, such as N, K, Mg, Fe, Mn, Cu, and Zn, which are useful as building blocks or in the chlorophyll biosynthesis process. On the other hand, EM4 contains photosynthetic and nitrogen-fixing bacteria thereby increasing the rate of plant photosynthesis, which is necessary to produce growth regulators (Joshi et al., 2019; Borowiak et al., 2021). These photosynthetic bacteria produce metabolites that will be used as an energy source

for the growth of other soil microorganisms (Abdulkareem et al., 2020) which are responsible for decomposing organic matter and releasing nutrients to be absorbed by plants (Spargo et al., 2013; Joshi et al., 2019).

The chlorophyll content which was higher with the application of EM4 than the control was also by the research conducted by Borowiak et al. (2021). EM4 also affects other growth substances, such as cytokinins, which also have a positive effect on chlorophyll biosynthesis (Borowiak et al., 2021). The effect of EM4 on the presence of auxin will affect the presence of cytokinins. Cytokinins are involved in the control of chloroplast biogenesis and function. They affect chloroplast and etioplast ultrastructure, chloroplast enzyme activities, pigment accumulation, and the rate of photosynthesis (Cortleven & Schmülling, 2015). Increasing cytokinin in cells will increase chlorophyll synthesis (Latifa et al., 2019; Borowiak et al., 2021).

Across all treatments, the average weekly pH of the soil was 7, which remained within the range that is favorable for cucumber plant growth. This can be caused by the administration of EM4 which contains lactic acid bacteria which produce lactic acid and yeast which produce alcohol from the decomposition process of soil organic matter. Lactic acid will produce H^+ for the soil and alcohol will produce OH^- (Maicas, 2020; Abdulkareem et al., 2020).

We observed the effect of EM4 on the growth parameters of cucumber plants. The positive impact of EM4 on the cucumber plant had been demonstrated in plant height, chlorophyll content, days to flower, number of flowers, and percentage of fruit set. This study also has shown that EM must be applied together with organic matter. They can be applied as a liquid, or mixed with nutrient-rich organic matter as manure. The benefit of applying EM plus organic matter lies in the ability of the EM to ferment organic matter, thereby releasing nutrients that can be used by plants

CONCLUSION

According to the research, it can be concluded that application of various EM4 doses had a significant effect on plant height, total chlorophyll, chlorophyll a, number of flowers, days to flower, and percentage of fruit set, but did not significantly affect chlorophyll b content. The best dose was shown at a dose of 40 ml EM4. For better cucumber growth EM4 application should be carried out in combination with manure and the recommended dose of NPK fertilizers.

ACKNOWLEDGMENTS

This research was partially funded by the MeCE (Metabolomics and Chemical Ecology) Research Group, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia. We also thank FMIPA Universitas Indonesia for facilitating the process of preparing manuscripts for publication.

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