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Applications of Mimosine Derivatives from Leucaena or SUBABUL plants as the Natural Bio-Herbicides

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ABSTRACT

Mimosine, a non-protein amino acid, is found in several tropical and subtropical plants, which has high value for medicine and agricultural chemicals. Here, research works aimed to development of natural product-based pesticidal agents, as we present the first significant findings for insecticidal and herbicidal activities of novel mimosine derivatives. Mimosine [α-amino-β-(3-hydroxy-4-oxo-1, 4-dihydropyridin-1-yl)-propanoic acid] is a major constituent of Leucaena (*Leucaena leucocephala* de Wit) and which is responsible for the strong allelopathic potential of the legume tree. Mimosine showed strong herbicidal activities on various plants in a bioassay. All plant parts of Leucaena contain mimosine. The quantity of mimosine in the young leaves and mature seeds was the greatest, 2.66 and 2.38% of dry weight, respectively, while the quantity in root xylems and xylems was the lowest: 0.18 and 0.11% of dry weight, respectively.

In this study, mimosine quantification of *Leucaena leucocephala* was carried out by using extraction and distillation methods which are soxhlet extraction with either distilled water or ethyl acetate as extraction solvent and digestion method were used to compare its efficiency in extracting the mimosine from *Leucaena leucocephala* leaves. In soil to which mimosine was added, about 60% of the mimosine was adsorbed in 1–5 days, and only a minor volume of mimosine was decomposed: 5.30 and 0.16% after 1 and 5 days, respectively. Mimosine has been evaluated as a major allelochemical in Leucaena, which is responsible for the strong allelopathic activity, and showed a suppressive impact on some tested plants and noxious fungi.

Keywords:- Mimosine, Leucaena, Toxicity, Herbicides / Bio-pesticide & Distillation / Extraction.

I. INTRODUCTION

Leucaena-leucocephala de Wit called "Subabul'' in India is a popular farm forestry tree in the coastal areas of Andhra Pradesh and Maharashtra. It is one of the fast growing hardy evergreen species in India it is shown in Figure (1). It is a vigorous coppiced and responds well to pollarding, lopping & pruning. It has deep and strong taproot and even the seedlings are deep rooted. There are four types of Leucaena-leucocephala.

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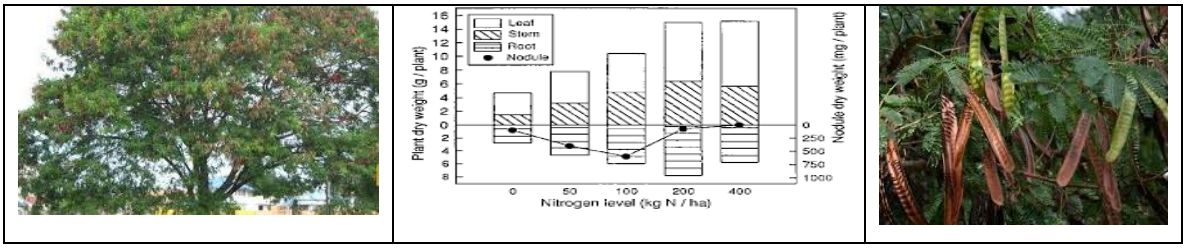

Figure (1):- Leucaena-leucocephala de wit (subabul).

Hawaiian Type: The plants are short bushy and remarkably drought tolerant. It is suited to hilly terrains in drought prone areas. It is a prolific seed producer and is good for fodder purpose.

Salvador Type: Tall, tree like and fast growing having maximum annual biomass production. Possesses large leaves, pods and seeds than Hawaiian types. Responds to high fertilization.

Peru Type: Tall and extensively branching type and is ideal for fodder purpose.

Cunningham Type: It is a cross between Salvador and Peru types.

Leucaena-leucocephala is best suited for warm regions and grows well between 22 and 30ºC in regions of 500 to 2000 mm annual rainfall. Because of its strong and deep root system, the tree is highly drought resistant. It is restricted to elevations below 500 m but withstands variations in rainfall, sunlight, windstorm, slight frost and drought. Leucaena-leucocephala wood can be used for light construction, poles, props, pulp, furniture, flooring and fuel wood. Leucaena-leucocephala wood is an excellent fuel wood with a specific gravity of 0.45 - 0.55 and a high heating value of 4000 kcal/kg. Leucaena-leucocephala forage has a high protein and carotene content and pellets or cubes are internationally marketed as animal feed. [1]

II. LITERATURE REVIEW

Pesticides make up one essential segment of the agro-business complex which is so vital to the future development of countries such as India. To emphasize the vital place of pesticides, it has been estimated that about 20 percent of food production in India may be lost due to destruction caused by various pests including rodents and weeds. Some of the newer organo-phosphorus compounds act as systemic insecticides which means they can be fed to plants or animals and thus effectively destroy pests at the moment of contact between the pest and the plant or animal; they need not be spread about externally, but are distributed by the inner circulatory systems of the plant or animal.[2] The classified pesticides with target pests comparison is shown Table [1].

Sr. No	Type Of Pesticide	Target Pest	Synthetic Pesticide	Natural Pesticide
1	Fungicides	Fungi	Mancozeb	Neem oil
2	Herbicides	Plants (Weed)	Glyphosate	Vinegar
3	Insecticides	Insects	Endosulphan	Salorra

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4	Nematicides	Nematodes	Carbofuran	Safin	
5	Avicides	Birds	Strychnine	Strychnine	
6	Rodenticides	Rodents	Zinc Phosphide	Strychnine	Commercial Pesticides
7	Acaricides	Spiders, mites	Fenazaquin	**Carvacrol**	
8	Algaecides	Algae	Dichlorophen	Barley Straw	
9	Bactericides	Bacteria	Validamycin		
10	Miticides	Mites	Abamectin	Avermectin	
11	Molluscides	Snails, slugs			
12	Piscicides	Fish	Rotenone	Barringtonia	

Table [1] - Types of pesticides and target pests.

Allelopathy is defined by Rice (1984) as the direct or indirect harmful or beneficial effects of the donor plant on the target one through the production of chemical compounds that escape into the environment. It includes both detrimental and beneficial interactions between plants through chemicals released by the donor.[3] However, in practice, the term allelopathy is generally used to refer to detrimental plant-plant interaction (Kohili et al., 1998).[4] The importance of allelopathy in agriculture is becoming increasingly recognized, in particular in the biological control of weeds and pests (Rice, 1984).[6] Allelopathy can be successfully utilized as a biological tool in weed and pest management by (1) the transfer of the production of allelopathic genes to crops, (2) enhancement of the production of natural toxins or their products as bio-pesticides through tissue or cell culture, and (3) the use of DNA recombinant technology. To date, very few achievements in (1) and (3) have been reported. Therefore the development of bio-pesticides for eco-friendly sustainable agricultural production is the most promising and feasible.[7].

Herbicides perform a vital role in the management of weeds. As the name indicates, herbicides are chemicals that kill or control vegetation. Although the ultimate effect of most herbicides is the same (usually death of weed), the way they control weeds is vastly different as listed in Table [1], with different commercial products. One or more of the vital processes must be disrupted in order for a herbicide to kill a weed. Herbicide enters plants through shoots, roots, other below ground organs and seed. The process of herbicide entry into treated plants is called absorption, which involves contact, penetration and movement of the chemical into the plant, whereas, adsorption is the attraction of ions or molecules to the surface of a solid. Many herbicides are applied to plant surfaces as foliar spray. Leaves are the primary means of herbicide entry through shoots, although herbicide absorption can also occur through other aerial organs such as substantial amounts of some herbicides are absorbed through stems or emerging coleoptiles.[8] Foliar herbicide absorption includes the following three steps which are represented in Figure (2); Retention of spray droplets on a leaf surface, Penetration of the herbicide into plant cells & Movement into the cytoplasm of the plant cell. Maximum retention occurs when leaves are positioned at 50° to 90° to the orientation to the incidence of the spray.[9]

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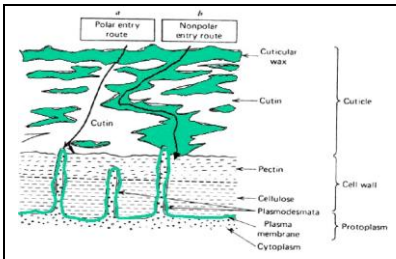
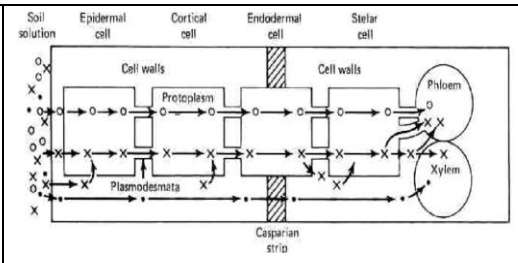
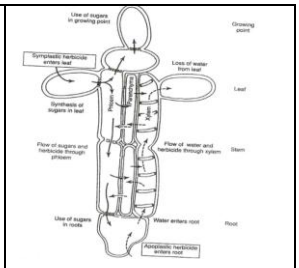


Absorption route from leaf surface to cytoplasm.



The herbicide absorption into roots.



Routes translocation of herbicides in plants.

Figure (2) :- Foliar herbicide absorption in root systems of target species.

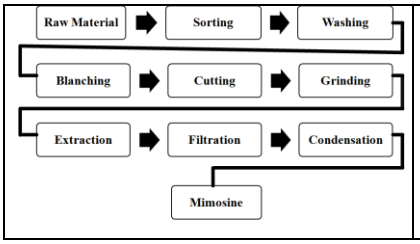
Herbicides that enter plants via the root hairs move upward in the xylem with the mass flow of water. Water and substances dissolved in water move in the xylem forward by transpirational pull and root pressure. The degree of translocation is often associated with the hydrophilic / lipophilic balance of the herbicide. In general, the movement in the xylem is faster than the phloem; therefore, when certain herbicides move readily between the symplast (phloem) and apoplast (xylem) their movement within the vascular system is in direct correlation with the transpirational stream.[10]

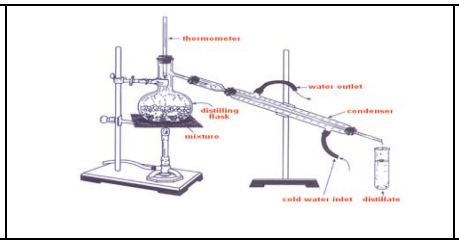
III. METHODS & MATERIALS

Extraction of mimosine is conducted at lab scale by using the leaves of Leucaena-leucocephala. The material and mythology follows to extracts the mimosine from the Leucaena-leucocephala de wit leaves is as follows.

Raw Material Required:- Leucaena-leucocephala de wit leaves, Distilled Water, Ethanol, Hydrochloric Acid, Ortho-phosphoric

Equipment Required :- Glass Kettle 3 litre Capacity, Pulveriser, Sieve 20 Mesh, 1000 ml size stoppered flask as shown in Figure (3), – Qty 01 Nos., 500 ml size stoppered flask – Qty 02 Nos. The constant temperature water bath, Buckler Filter, pH meter, Vacuum Pump and HPLC.[11 & 12]





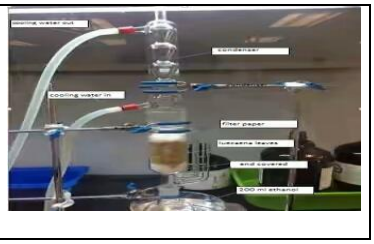


Figure (3):- Process FLOW Chart with the main diagram of Distillation & Extraction Unit.

Methods of Extraction; Extraction of mimosine by using distilled water and ethanol, Extraction of mimosine by using distilled water and ethanol. The extraction of mimosine from leucaena leaves is carried out as; Collected 1 kg of healthy leaves of Leucaena leucocephala, allow drying the leaves at 39 to 40 °C, grinding or pulverising dried leaves in powder form by using pulveriser and sieve it from 20 mesh sieve. Then take 500 ml distilled water in 1000 ml size stoppered flask, and then adds the 100 gram leaves powder in the distilled water. Place the flask in constant temperature water bath, and allow standing for extraction for 16-17 hrs at 59°C~60

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°C. After complete extraction filter the mass through centrifuge and collect supernatant in another 1000 ml size flask. Then adjust the pH of supernatant with hydrochloric acid (2mol / L) at pH 5 with pH meter. Charge ethanol in this solution and allow to maintain at 4°C for two days. Filter the mass through centrifuge (6000r / min), collect the supernatant. Concentrate the supernatant by using vacuum pump at 4°C so that final material remains at volume of 20 ml only and allow to stand this mixture for 3 days. Process Parameter, observation table for mimosine extract concentration temperature for batch-1 & 2, the Material Balance for Batch-1 & 2 are reported by Pund, G.V.[8]

We have examined various conditions for mimosine purification and observed that the type of ion exchange resin and adjustment of pH are crucial conditions to obtaining the maximum quantity and high purity of mimosine (5 g per 1 kg fresh Leucaena leaves, purity>95%). Mimosine is considered as an allelochemical and is responsible for the allelopathic activity of the *Leucaena* genus and other species belonging to *Mimosa* spp. Leucaena is popular in intercropping with annual crops, using as a hedgerow, and alley cropping for yield promotion and weed control.[14 & 15] The Chemical Structure of mimosine is shown in Figure (4).

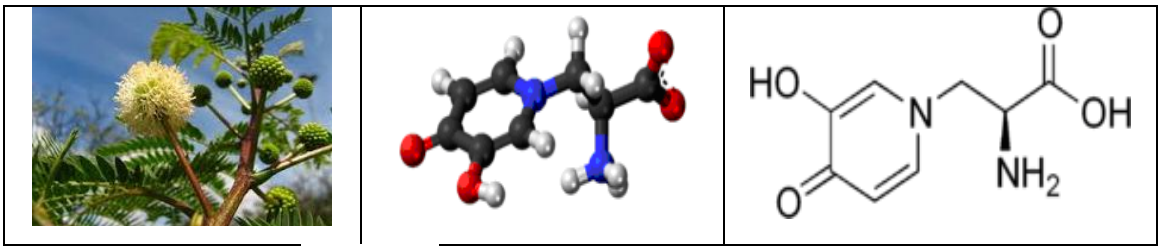

Figure (4):- Chemical Structure of mimosine.

Mimosine or leucenol is an alkaloid, β-3-hydroxy-4 pyridone amino acid. It is a toxic non-protein free amino acid otherwise chemically similar to tyrosine, and was first isolated from Mimosa pudica. It occurs in a few other Mimosa spp. and all members of the closely related genus Leucaena.[16]

Properties of Mimosine :- Chemical formula - $C_8H_{10}N_2O_4$, Molecular weight / Molar mass :- 198.18g·mol^{-1}, Melting point :- 291°C (556°F; 564 K).[17] Content of Mimosine in Different Part of Leucaena Plant was found to be as; Xylems – 0.11%, Xylems roots – 0.18%, Mature leaves – 0.47%, Mature Stems - 0.54%, Hairy Roots – 0.66%, Cortex of roots – 0.66%, Mature seed pods – 0.67%, Bark – 0.68%, Immature seed pods – 0.88%, Immature seeds – 1%, Flowers 1.17&, Flower buds – 1.34%, Young stems – 1.5%, Mature seeds – 2.38%, Young leaves – 2.66%.[13].

IV. ANALYSIS

Mimosine extracted at lab scale is further analyzed by using HPLC method. A useful HPLC system having 880-PU pump and column Fine peak Sil C18 of Nihonbunko company is used to determine mimosine and DHP contents by using a solvent system of 0.2% ortho-phosphoric acid at wavelength of 280 nm, the peaks of mimosine and DHP were detected at retention time of 2.5 min and 7.4 min, respectively. HPLC analysis report of mimosine extracted by using water and ethanol in batch no-1. Similarly HPLC analysis report of mimosine

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extracted by using ethanol in batch no-2, shown in Figure (5) below In analysis report the peak of mimosine and DHP are clearly seen at the retention time of 2.595 min and 7.308 min respectively.

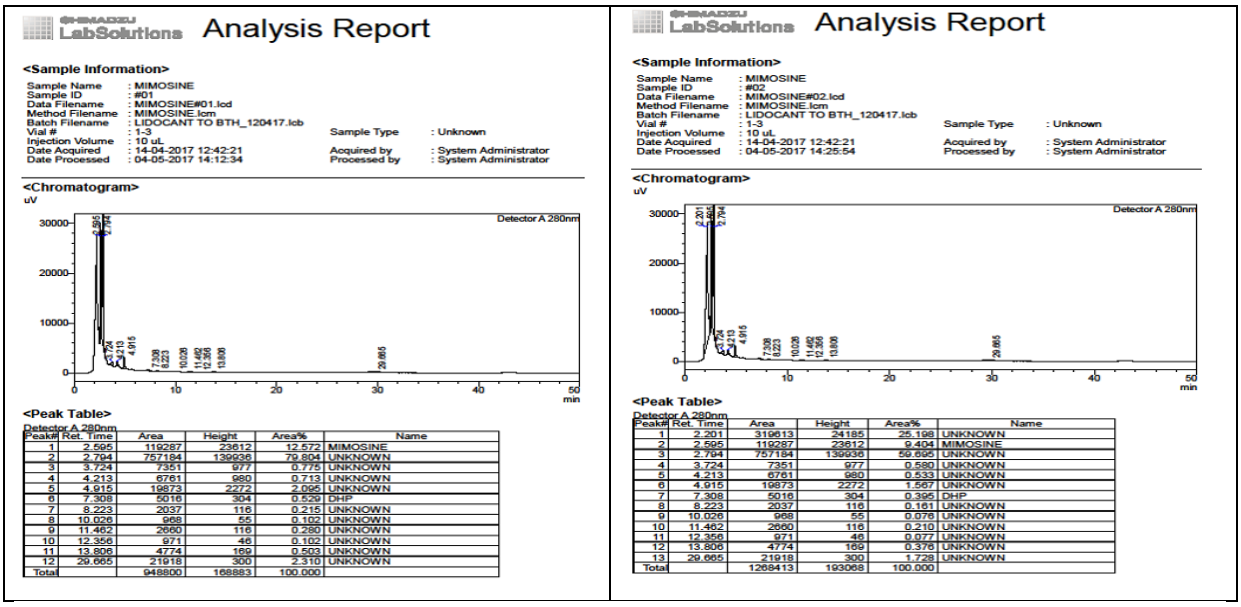

Figure (5):- HPLC analysis report for batch number 1 & 2.

In analysis report of batch no-2, the peak appears at residence time 2.201min is the peak for impurity which is not found in analysis report if batch no-1 Other impurities in both of the batches are common and unknown at retention time of 2.794 min, 3.724 min, 4.213 min, 4.213 min, 4.915 min, 8.223 min, 10.026 min, 11.462 min, 12.356 min, 13.806 min, 29.662 min. From the comparative study of both reports, it is observed that the first impurity at retention time of 2.201 min is completely washed out in batch no-1. Select 550 mm wavelength spectrometer analysis, the extracted mimosa prime purity of 99%. Each 100g Leucaena leaves can be extracted mimosa prime 2.1~2.4g, press Leucaena leaves (Mimosa) of mimosa pigment content of 3% ~ 6%, the extraction rate of the method is 40% ~ 70%. [11].

V. RESULT & DISCUSSIONS

The mimosine extract extracted at lab scale by using leucaena leaves, are applied on the soil used for cultivation of mimosine. The study is carried out for the 7 days. At first day of cultivation approximately 100 no of mustard seeds are cultivated in five different containers. All five containers cultivated with mustard seed, are applied with mimosine (250 ml extract) with different concentration. Water is added for the cultivated mustard with gap of one day in each container. All container containing mustard seeds, i.e. container-1, container-2, container-3, are treated as a sample of testing i.e. sample-1, sample-2, sample-3 respectively. From the following Figure (6) is clear that, the concentration of mimosine extract is directly affect the emergence of mustard seed. The container-1 is applied by the 250 ml of mimosine extract with a concentration of 100 mg/liter, where 73 numbers of mustard seeds are emerge out against approximately 100 mustard seeds. Similarly at the concentration of 1000 mg/liter concentration only 6 seeds are emerge out within the 7 days of their life.

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			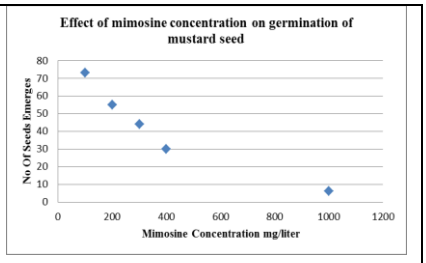
73 no's of mustard seeds emerge out for 100mg/liter.	55 no's of mustard seeds emerge out for 200mg/liter.	44 no's of mustard seeds emerge out for 300mg/lit.	Graph showing mimosine concentration Vs mustard seed emergence.

Figure (6):- Mustard seeds emergence and concentrations of mimosine.

Thus the mimosine is act as a pre-emergence herbicide for mustard seeds; hence it is called as Seedling Growth Inhibitors which inhibit the emergence power of seed by affecting the roots of the mustard seed. Hence mimosine can be used as selective herbicide over the wild mustard plant, which may act as a weed to the main plant. Mimosine showed strong herbicidal activities on various plants in a bioassay as shown in Figure (7).

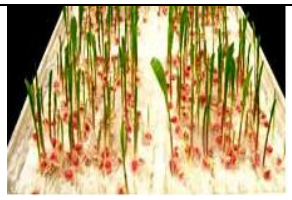			
Warm Standard Germination Duration 7-10 days.	Sand Germination Duration 7-10 days.	Cold Germination, Duration 12 -14 days.	Saturated Cold Germination, duration 9 - 10 days.

Figure (7):- Mimosine showed strong herbicidal activities on various plants in a bioassay.

Warm Standard germination tests are used for labeling purposes and give a reasonable idea of field emergence under favorable conditions. **Sand germination tests** are done the same as Warm germination, This test is useful in suppressing some fungi. It also aids in uniform uptake of water, especially in low moisture soybean seed. For soybean samples, sand germination results are the same or slightly higher than warm germination results. **The Cold germination test** gives a reasonable idea of emergence under less than ideal conditions. **The Saturated Cold germination test** is another way of determining how well a seed lot will do under unfavorable conditions. Similar to other phytotoxins, effects of mimosine against plant germination and growth are proportional to applied doses. Mimosine also shows selective influence against certain bacteria and fungal growth. Some bacteria were inhibited, whereas growth of several bacteria was promoted by mimosine.

VI. CONCLUSIONS

The findings in this study repeatedly demonstrated that mimosine is responsible for the allelopathic activity in Leucaena. This non-protein amino acid exhibited a strong reaction to various plants and weeds, suggesting that this compound may be utilized as a leading herbicidal compound for the development of bioactive herbicides.

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Momosine is extracted by using different sources of legume tree, like mimosa pudica, leucaena-leucocephala. Mimosine is found in different part of Leucaena leaves, but can be extracted through leaves easily. The analysis of Extracted mimosine is determined by using HPLC method. Mimosine is extracted in two batches at lab scale. i.e. Batch no-1 using water and methanol solvent has a relatively more pure than the batch no-2 in which only ethanol is used as a solvent. Extraction process followed in batch no-2, is quite easy than batch no-1, because supernatant obtained in batch no-2 is more clear than the batch no-1, because of separation of water is difficult through lab scale filtration process in batch no-1. One of the unknown impurity at retention time 2.201 min which is not appeared in batch no-1 may be water insoluble. Mimosine is act as herbicide for mustard seeds. Mimosine is act as seeding growth inhibitor or mustard seed. Concentration of spray of mimosine extract is directly affecting the seed germination. Approximately 1.5 to 2 grams of mimosine can be extracted from 100 grams of Leucaena leaves. Mimosine is a non-protein amino acid and it may be easily degraded after penetrating into soils by soil factors such as nutrients, minerals, pH and microbes. Therefore, Mimosine in leucaena as a potent bio-herbicide examining the fate of mimosine in different soils measured by standard kinetic parameters should be carried out in future research to estimate the potential use of mimosine as a bio-pesticide in agricultural practices.

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