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PHYTOTOXIC ACTIVITY OF *MELIA AZEDARACH*(L.) AQUEOUS LEAF EXTRACTS ON GERMINATION AND SEEDLING DEVELOPMENT OF *ABELMOSCHUS ESCULANTUS* (L.) AND *SESAMUM INDICUM* (L.)

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ABSTRACT

Phytochemicals are secondary metabolites produced by a variety of plants that can prevent competitor species from germinating. Allelopathy is the name for this process, mediated by various substances, the most common of which are phenolic compounds. Plant allelochemicals can thus be employed in agricultural systems to suppress weeds and crops. This study aimed to determine how *Melia azedarach* (L.) aqueous leaf extracts affected *Sesamum indicum* (L.) and *Abelmoschus esculentus* (L.) seedlings in terms of germination, seed index, seedling length, and biochemicals of crops. Aqueous extract of *Melia azedarach* L. leaves was assayed at 10%, 20%, 40%, 60 %, and 80% concentration on germination and initial growth of sesame and okra in pot culture. However, the results of a pot experiment with test crops revealed that leaf extract had stimulatory and inhibitory phytotoxic effects on germination, seedling growth, and biochemicals compared to control. Depending on the plant species and extract content, the efficiency of these inhibitory actions differed.

Keywords: Phytotoxic, germination, vigour index, *Melia azedarach*, *Abelmoschus esculentus*, *Sesamum indicum*

INTRODUCTION

Allelopathy is a biological phenomenon in which a plant species produces secondary metabolic chemical substances that affect the growth, survival, and development of other plant species[1]. Secondary metabolites, also known as allelochemicals, are released to induce effect[2-4]. Allelochemicals induce unfavorable physiological effects in neighboring plants, such as inhibiting the percentage and speed of germination and reducing the initial growth of seedlings[5]. Allelochemicals are natural phytotoxins that, as an alternative to commercial herbicides, can manage weeds in sustainable agro-ecosystems.

Synthetic materials are generally discouraged due to their high cost and environmental issues associated with their use. To maintain biosafety and environmental sustainability, scientists are developing novel environmental solutions for plant growth augmentation[6]. Using allelopathic components as bioherbicides in agricultural systems can have many advantages over the usual synthetic products[7-8]. Phytotoxic action has been demonstrated for several chemicals, including phenolics and their breakdown products. Seed sprouting and root growth are both significantly hampered by their inhibitory actions[9]. Phenolic compounds, which include phenols, flavonoids, hydroxycinnamic and benzoic acids, phenylpropanoids, coumarins, and tannins, are a prominent group of allelochemicals. Different plant parts, including flowers, leaves, leaf litter, and leaf mulch, stems, bark, roots, soil, and soil leachates and their derived compounds, can have an allelopathic activity that varies over a growing season. Various plant species produce phytochemicals, and their anti-weed and anti-crop activities have been widely established[10,11].

The most important pathway of releasing allelochemicals is root exudation. By interacting with inorganic and organic soil components and soil microorganisms, root exudates impact the bioavailability and phytotoxicity of allelochemicals[12]. The prevailing soil conditions and the physical, chemical, and biological transformations of allelochemicals in the soil environment determine the phytotoxic levels of these compounds in the soil[13].

Allelopathy supplies a less laborious and effective biological alternative to chemical and mechanical weed control methods without affecting the environment adversely[14]. It has presented an alternative for developing eco-friendly agricultural practices, enhancing crop productivity, and maintaining ecosystem stability[15]. Moreover, allelopathic interactions may be significant in ecosystems by influencing weed control and crop productivity[16].

Melia azedarach L. is a Meliaceae family commercial perennial deciduous tree. A fast-growing multipurpose tree goes by several names, including Indian lilac, Persian lilac, white cedar, chinaberry tree, bead tree, Cape lilac, and Syringa berry tree. It is preferred in the alley cropping system of agroforestry and ornamental purposes [17]. It is planted by farmers in either block or boundary plantation agroforestry systems. It also aids in soil fertility improvement[18]. The species' industrial and ecological importance has prompted farmers to establish large-scale plantations with various intercrops, including shade trees in coffee and abaca (Musa textilis) plantations, sugarcane and other vegetables, legumes, and grain crops[19]. Studies on the allelopathic effects of leaf extracts and leaf litter on the growth and yield of understorey crops are required to create and market *M. azedarach* based tree-crop combinations. According to the current literature, there is few research on this significant species. As a result, the present study was undertaken to investigate the allelopathic effect of *M. sylvestris* leaf aqueous extracts. In a pot culture experiment, *M. azedarach* was used to study the germination, seedling growth, vigour index, and biochemical analysis of *S.indicum* and *A.esculantus*.

MATERIALS AND METHODS

Study Area

The present study was carried out in the green house complex of Rani Anna Government College for women, Tirunelveli, Tamilnadu, India. The study site is located at 8.7139° N latitude 77.7567° E longitude.

Collection of plant material

The fresh matured leaves of *Melia azedarach* were collected from 5 year old plantations during October 2020 at Chettikulam village of Tirunelveli District, Tamil Nadu, India. The leaves of the plant were washed, cut into pieces and allowed to dry in the shade. The dried plant materials were grounded in a mixer grinder and stored in air-tight glass bottles after sieving.

Collection of Seeds

The viable seeds of *S. indicum* and *A. esculantus* were collected from the local market of Tirunelveli District of Tamilnadu, India.

Preparation of Plant extract

100 g of powdered dried leaf powder was soaked in 1L distilled water to make aqueous extracts. For 24 hours, the solution was mixed and stored at ambient temperature (20-25°C). The extract was filtered through a sieve and considered as 100% stock for further analysis. By diluting the stock with distilled water, other concentrations such as 10%, 20%, 40%, 60%, and 80% were prepared. The control was distilled water without the plant extract. In a completely randomized approach, the treatments were reproduced five times (CRD)

Pot Experiments

The effects of *M. azedarach* leaf extracts on germination, initial growth, vigour index, and biochemical contents of both test crops were investigated in pot tests conducted in a greenhouse. Total twenty seeds of each test crop were sown on soil filled in the earthen pots [16 cm diameter x 18 cm height] containing garden soil (N, P and K content 84.82, 17.85 and 80.35 ppm, respectively). Leaf extract was applied at 10%, 20%, 40%, 60% and 80% and the control pot was treated with distilled water. They repeated each procedure three times. After 10 days of seed sowing, germination was recorded. After 15 days after sowing, the seedlings were uprooted and washed with tap water to remove any soil left on the roots. After that, for biophysical and biochemical parameters, the seedlings were examined.

Germination Percentage

The percentage of germination was calculated as follows. Germination % = No. of seeds germinated/ Total no. of seeds sown × 100

Growth of the Seedlings

The growth of the seedlings was estimated by measuring the shoot and root length of 5 randomly selected plants, and the average values were taken as cm/plant

Seedling Vigour Index

The vigour index of the seedling was calculated as follows. SVI= Germination (%) × [shoot length (cm) + root length (cm)]

Statistical Analysis

All the experiments were carried out in triplicates and carried out separately three times with consistent findings. Data is defined as the mean of replicates ± SD. Differences between treatments were then tested using one way-variance analysis (ANOVA) for each parameter under review and followed by Duncan's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Table :1. Phytotoxic potential of *M. azedarach* on germination percentage of *A. esculantus* and *S. indicum.*

Extract Concentration	*A.esculantus*	*S. indicum*
Control	87	90
10%	88 (1.15)	93 (3.33)
20%	90 (3.45)	87 (-3.33)
40%	93 (6.89)	70 (-24.73)
60%	84 (-3.44)	50 (-42.53)
80%	65 (-25.29)	31 (-65.55)

- Data in parenthesis indicates %increase/decrease over control

In the current investigation, practically all extracts significantly lowered and induced seed germination percentages in two types of seeds when compared to the control. In the seeds of *A. esculantus*, the concentrations of *M. azedarach* extract treatment the germination percentage was increased significantly except at 60% and 80%, compared to the control. For *S. indicum*, the germination percentage was reduced significantly in all the *M. azedarach* extract treatment concentrations compared to control. The stimulatory and inhibitory effects of germination of species are different and depending on the concentration of extracts.

Germination percentage is considered to be an excellent indicator for the detection of allelopathic potential[20]. The seed germination process is complex and involves sequential changes in the biochemistry, physiology, and morphology of the seedlings. The leaf extracts of *Conocarpus lancifolius* held up germination and growth of *Vigna sinensis* L. and *Zea mays* L. in a concentration dependent manner[21]. The leaf extracts of *Brassica nigra* *Eucalyptus camaldulensis* , *Acacia auriculiformis* , and alfalfa inhibited the germination and growth of some crops[22,23]. Phytochemicals are thought to hinder seed germination by disrupting mitochondrial respiration and oxidative pentose phosphate pathways, according to a previous study[24]. Furthermore, the synergistic effect of multiple phenolic compounds contained in the extract may increase phytotoxicity[25]. The inhibitory activity on germination was, however, depending on the test plant species and concentration.

Table-2 Phytotoxic potential of *M. azedarach* on root length (cm) and shoot length (cm) of *A. esculantus* and *S. indicum*.

Extract Concentration	*A.esculantus* Root length	*A.esculantus* Shoot length	*S. indicum* Root length	*S. indicum* Shoot length
Control	9.2	23.4	8.2	20.2
10%	10.3 (11.95)	24.2 (3.41)	7.9 (-3.66)	19.1 (-5.44)
20%	11.2 (21.74)	26.5 (13.25)	6.8 (-17.07)	17.5 (-13.3)
40%	12.5 (35.87)	27.8 (18.80)	5.6 (-31.71)	15.8 (-21.78)
60%	8.4 (-8.69)	20.1 (-14.10)	4.3 (-47.56)	11.8 (-41.58)
80%	5.8 (-36.96)	12.2 (-47.86)	2.6 (-68.29)	7.8(-61.38)

- Data in parenthesis indicates %increase/decrease over control

The root length and shoot length of the two test seedlings were affected by the aqueous leaf extract treatment of *Melia azedarach* (Table -2). The lowest root length and shoot length values were recorded in *S. indicum* and the highest values were noticed in *A. esculantus*. It may be the presence of a number of phytochemicals i.e., terpenoids, flavonoids, steroids, acids, anthraquinones, alkaloids, saponins, tannins etc. in the aqueous leaf extract of *M. azedarach*. Leaves contain terpenoids and limonoids like l-Cinnamoyl-3-acetyl-11-hydroxy meliacarpin, l-Cinnamoyl3-methacrylyl-l1-hydroxy meliacarpin, Deacetyl salannin, 1,3-Dicinnamoyl-l1-hydroxy-rneliacarpin, α-Pinene, β-Pinene, αTerpinene, α-Terpineol,Kaempferol-3-O-β-rutinoside, Kaempferol-3-L-rhamno-D-glucoside, Rutin. They also contain acids like Palmitic acid (hexadecanoic acid). The extract treated *A. esculantus* showed characteristic variations in their root length and shoot length compared to the control plants. At higher concentrations treatment (60% and 80%), root length and shoot length were significantly reduced compared to the control. Furthermore *S. indicum* was highly affected by *M. azedarach* extract in all the concentrations compared to the control. The inhibition was found to be concentration and species-dependent manner. Some seedlings showed a high specific root length than the shoot length, and others had stunted roots. Reports suggested that these are characteristics of plants growing in sterile environments or exposed to environmental stress[26]. *Prosopis juliflora* (Sw.) DC. aqueous leaf extracts were found to have substantial inhibitory effects on the root length of *Triticum aestivum* L. in a study[27]. The WE of *Eclipta alba* (L.) leaves inhibited the root radicle length and stem length of *C. tora* and *C. sophera* in another investigation[28]. Phenolic compounds are the most widely documented metabolites in higher plants that have a function in defense systems. They target plant cellular activities at different places with varying levels of toxicity[29]. Caffeic acid compounds from *Bellis perennis* L. were found to exhibit possible allelopathic properties in prior research. When it came to the growth of herbal species, differences were depending on inhibitory effects on some plants (e.g., *Dactylis hispanica* Roth) and stimulatory effects on others (e.g., *Aegilops geniculate* Roth)[30]. The leaf extracts of *Gliricidia sepium* inhibited the growth of lettuce radicles due to the presence of coumarins[31].

Table-3 Phytotoxic influence of *M. azedarach* on Vigour Index of *A. esculantus* and *S. indicum*

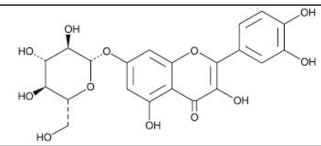
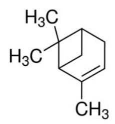
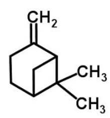
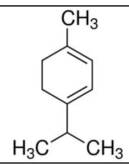
Extract Concentration	*A.esculantus*	*S. indicum*
Control	2836.2	2556
10%	3036 (7.04)	2511 (-1.76)
20%	3393 (19.63)	2114.1 (-17.29)
40%	3747.9 (32.14)	1498 (-41.39)
60%	2394 (-15.59)	805 (-68.51)
80%	1170 (-58.75)	322.4 (-87.39)

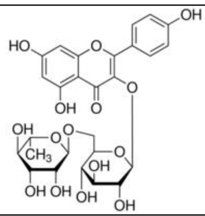
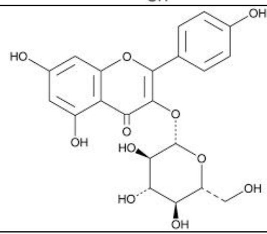
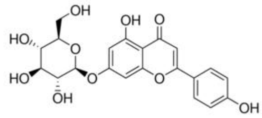
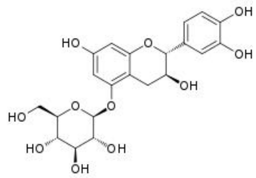
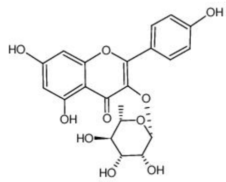
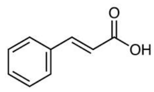
- Data in parenthesis indicates %increase/decrease over control

M. azedarach extract had a significant stimulatory effect of Vigour Index in *A. esculantus* and inhibitory effect in *S. indicum* on various parameters studied compared to the control. It may be the influence of phytotoxic compounds which are present in leaf extract of *M. azedarach*. The main phytochemicals are uercetin ,rutin ,catechin, kaempferol, kaempferol-3-O-glycoside, quercetin-7-O-glycoside, apigenin-7-O-glycoside,kaempferol-7-O-glycoside,catechin-5-O-glycoside and flavonoids like 4', 5-Dihydroxy flavone-7-O-u-L-rhamnopyranosyl-(1-4)-β-D-glucopyranoside, Anthraquinone like 1,3,5,8-Tetrahydroxy-2-methyl anthraquinone; 8-Me ether, 3-O-α-L-rhamnopyranoside, 1,5-dihydroxy-8- methoxy-2-methl-anthraquinone- 3-O-α-L-rhamnopyranoside, 1,8- dihydroxy-2-methyl anthraquinone-3-O-β-D, galactopyranoside. Acids like Stearic acid (octadecanoic acid), Trans-cinnamic acid. Steroids like 24-Methylenecydoartanol, 24-Methylenecydoartanone, 4- Stigmastanen-3-one, 4-Campestene-3-one β-Sitosterol, βSitosterol-B-D-glucoside etc. These phytochemicals inhibited the growth of seedlings at higher concentration treatment more than the lower concentration treatment over control.

At Low concentrations treatment (10%, 20% and 40%), the phytochemicals which are available in the extract also low of uercetin ,rutin ,catechin, kaempferol, kaempferol-3-O-glycoside, quercetin-7-O-glycoside, apigenin-7-O-glycoside,kaempferol-7-O-glycoside,catechin-5-O-glycoside and flavonoids like 4', 5-Dihydroxy flavone-7-O-u-L-rhamnopyranosyl-(1-4)-β-D-glucopyranoside, Anthraquinone. These chemicals can increase cell division and enlargement by accelerating mitosis and cellulose synthesis in the *A. esculantus* seedlings. At the same time the lower concentration also affected the *S. indicum* seedlings vigour index. Low quantities of benzoic, coumaric, gallic, and caffeic acids have been shown to enhance cell division and enlargement by speeding up mitosis and cellulose synthesis[32], allowing maize seedlings to grow faster.

Table : 4 List of some compounds

S.No	Compound Name	Compound Structure
1.	l quercetin-7-O-glycoside	
2.	α-Pinene,	
3	β-Pinene	
4.	αTerpinene,	

5	α-Terpineol,Kaempferol-3-O-β-rutinoside	
6	Kaempferol-3-L-rhamno-D-glucoside	
7	Palmitic acid	$CH_3(CH_2)_{14}-\overset{O}{\overset{\|\|}{C}}-OH$
8	apigenin-7-O-glycoside	
9	catechin-5-O-glycoside	
10	8-Me ether, 3-O-α-L-rhamnopyranoside,	
11	trans-cinnamic acid	

After 15 days of treatment, the extracts from the fresh leaves of the *M. azedarach* decreased the biochemical constituents of both test seedlings, i.e, starch, protein and amino acid [Fig 1 & 2]. From 10% to 40% extract treatment in *A. esculantus* found that significantly increased starch, protein and amino acid (starch: 1.39%, 3.00% and 4.72% respectively, protein:0.82%, 1.85% and 6.95% respectively, aminoacid : 0.89%, 3.23% and 8.03% respectively) and 60% & 80% extract treatment of seedlings noticed reduced starch, protein and amino acid contents (starch: -9.65% and -39.16% Protein : -13.55% and -47.96%, aminoacid:-7.25% and -24.66%). In *S. indicum* seedlings all the concentrations of *M. azedarach* extract treatment only reduction was noticed. Less reduction percentage was noticed in less concentration of extract. At a high leaf extract concentration (60% &80%), more reduction percentage was noticed in starch, protein and amino acid. More reduction percentage was noticed in aminoacid, starch and followed by protein in *S. indicum* seedlings. Because the effect of the test seedlings was concentration and species-dependent. The presence of phenolic chemicals, which aid in the assimilation of amino acids into structural protein-based building blocks in the plant, may also increase protein content[33].Uercetin ,rutin ,catechin, kaempferol, kaempferol-3-O-glycoside, quercetin-7-O-glycoside, apigenin-7-O-glycoside,kaempferol-7-O-glycoside,catechin-5-O-glycoside and flavonoids can be present in allelopathic extracts.When used at low concentrations, they help prevent the development of reactive oxygen species (ROS). Applying aqueous extracts containing p-coumaric acid at low concentrations, on the other hand, can strongly activate indolacetic acid (IAA). Cinnamic acid at low concentrations can prevent IAA from degradation and promote seedling growth. Phenolic compounds, having various functions, act predominantly as antioxidant agents and reduce the harmful effects of reactive oxygen species[34]. The results showed that the climatic conditions and the type of solvent could significantly affect the phytochemical composition of a plant extract. The quantity of polyphenols seems to be related to climatic variations. In warmer climates, phenolic compounds usually increase, primarily due to the enhancement of photosynthetic activity[35]. The results revealed that notable inhibitory effects on *S. indicum*.

Fig: 1 Phytotoxic potential of *M. azedarach* extract on starch, protein and amino acid (mg/g.fr.wt) constituents of *A. esculantus* seedlings.

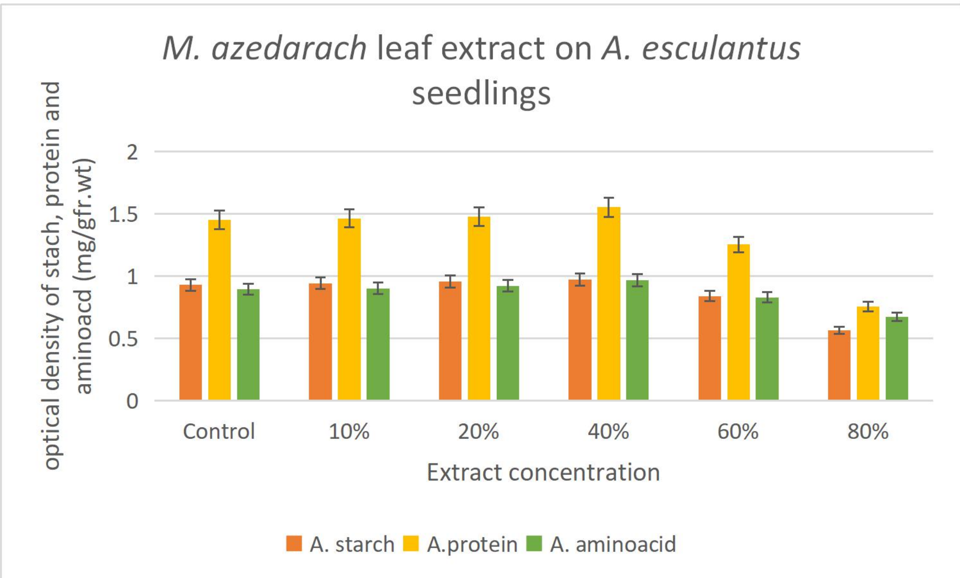
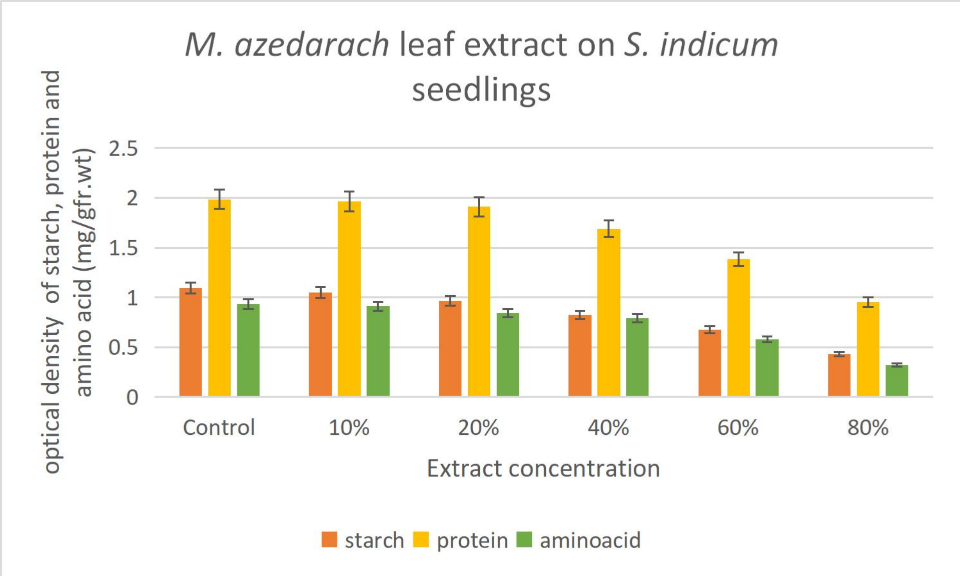


Fig-2 Phytotoxic potential of *M. azedarach* extract on starch, protein and amino acid (mg/g.fr.wt) constituents of *S. indicum* seedlings



CONCLUSION

Phytochemicals are essential in investigating appropriate farming systems, controlling weeds, diseases, and insects, alleviating continuous cropping obstacles, and allelopathic cultivar breeding. Furthermore, allelochemicals can act as environmentally friendly herbicides, fungicides, insecticides, and plant growth regulators and can have great value in sustainable agriculture. The results from the study indicated that *M. azedarach* exhibited stimulatory effects on the vegetative growth of *A. esculantus* and beneficial functions of the allelochemicals in *M. azedarach* leaf extract. Therefore, *M. azedarach* can be employed to increase the germination and seedling growth of *A. esculantus* and, in turn, increase its productivity at lower concentrations. At the same time, the *S. indicum* seedlings showed only an inhibitory effect on *M. azedarach* extract treatment in all concentrations. These results confirmed the phytochemicals' inhibitory and beneficial functions in *M. azedarach* leaf extracts at different concentrations. High concentrations of extracts yielded the worst effects but, in many cases, acted in a species-specific manner. The study demonstrated that water-soluble inhibitory and promotory substances were present within the leaf extracts of *M. azedarach*. The soluble substances can significantly affect the germination and growth of *Abelmoschus esculentus* and *Sesamum indicum*. The results suggest that *M. azedarach*'s fresh biomass could be used in the vegetable field of *Abelmoschus esculentus* as green manure, increasing germination and growth and developing the seedlings. Different variations in the components of *M. azedarach* extracts can be valuable from a phytotoxic point of view to formulate natural herbicides. Further study is recommended to identify the exact allelochemicals in the plant which promote crop growth or inhibit crop growth.

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