

Conocarpus Mediated Improvement in Photosynthetic Pigments of *Cicer arietinum* Growing Under Copper Stress

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ABSTRACT

Present work was planned to investigate the improvement in photosynthetic pigments of *Cicer arietinum* by *Conocarpus lancifolius* growing under copper stress. *Conocarpus lancifolius* was chosen as a biosorbent because it is abundantly and easily available plant of Province Sindh. It does not require so much efforts or money or even water to cultivate them, moreover, it is a fast grower. Plants were grown in randomized block design in triplicates. Each pot was supplied with 1kg of soil, the control plants were supplied with distilled water only, experimental plants were supplied with different concentration of copper viz. 0, 25, 50, 75 and 100 ppm whereas treated plants were supplied with 5gm *Conocarpus lancifolius* leaf powder along with the different concentration of copper. Effect of Copper on *Cicer arietinum* and its biosorption by *Conocarpus lancifolius* was observed by the determination of photosynthetic pigments (chlorophyll a, b, total chlorophylls and carotenoids) and its products (glucose, sucrose and total soluble sugars). Various vegetative growth parameters were also analyzed to correlate and confirm our results like % germination, root and shoot lengths, leaf area and relative water content. *C. lancifolius* was found not only an efficient, eco-friendly and cost-effective biosorbent for heavy metal copper but it is also an effective fertilizer too.

Keywords:

Conocarpus lancifolius, Copper, Biosorption, Photosynthetic pigments, *Cicer arietinum*.

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INTRODUCTION

Conocarpus lancifolius is commonly known for its unfriendly traits like the unpleasant smell, pollen allergy, and its roots can cause damage to walls. Chemical compounds which are present in *C. lancifolius* are Flavonoids, Saponins, and phenols^{1,2}. The tree's wood is dense and suitable for charcoal. Goats use the young trees and shoots as fodder, although the leaves contain tannin³. Because of its high salt tolerance and relative drought tolerance, the tree is sometimes planted as a pioneer species in reforestation projects in its native habitat. Copper toxicity has become a threat to both

agricultural and environmental concerns. Usual sources of Copper contamination in agricultural soil include industrial effluents, agricultural wastes, and the use of agrochemicals like insecticides, fungicides, and pesticides. Copper is present in many forms in soils, with free Cu²⁺ activity considered to be the best indicator of phytoavailability⁴. Adsorption of Cu is highly pH dependent and phytoavailability of Cu increases with decreasing pH⁵. Soluble Cu accounts for only a very small proportion of the total amount in the soil. Copper has a high affinity for solid phase organic matter, and is

therefore not readily leached and can accumulate in the surface soil^{6,7}. Copper is an essential micronutrient but exposure to excess Cu has a detrimental effect on plant growth leading to cellular damage of plants^{2,8,9}. This leads to the inactivation of biomolecules by blocking essential functional groups or by displacing the essential metal ions¹⁰. The effect of Cu toxicity is largely on root growth and morphology. Copper tends to accumulate in the root tissue with little translocation to the shoots¹¹. It can kill bacteria, algae, plants, snails, and fungi¹².

The current research was based on the question that could *Conocarpus lancifolius* improve the photosynthesis in *Cicer arietinum* growing under copper stress?

MATERIALS AND METHODS

Good quality seeds of *Cicer arietinum* were purchased from local market, healthy seeds were selected and were surface sterilized. Total 27 soil bags were prepared each provided with 1 kg of garden soil. Among them, 3 were marked as control supplied with only distilled water. 12 soil bags (triplicates of each Cu concentration i.e., 25ppm, 50ppm, 75ppm, 100ppm) were marked as experimental plants. 12 soil bags (triplicates of each Cu concentration) were marked as treated plants, also supplied with 5 gm/Kg of *Conocarpus lancifolius* powder as a biosorbent. Seeds were carefully sown (6 seeds/soil bag). After 20 days of sowing physical parameters like percent germination root, shoot length, leaf area, and relative water content was observed. Biochemical tests on the subject of chlorophyll contents were performed by¹³ and¹⁴ method, carotenoids by¹⁵, protein contents by¹⁶ and glucose, sucrose, total soluble sugars by¹⁷ were estimated.

RESULTS

The current research was designed to evaluate the capabilities of *Conocarpus lancifolius* on photosynthetic pigments of *Cicer arietinum* growing under copper stress. Along with photosynthetic pigments, several other biophysical and biochemical parameters were analyzed, which are given below.

Rate of germination

The rate of germination of *Cicer arietinum* was found to be 44, 48, 34 and 35% respectively growing under copper amended soil, whereas it was 40% in control plants. Low concentrations of copper are thought to be beneficial to plants¹⁸, that is why the rate of germination was found to be increased Table 1. Adverse effects were found on the rate of germination of *Cicer arietinum* at higher concentrations of copper (75 & 100 ppm), which may attribute to the augmentation of protease activity and reduced amylase activities¹⁹⁻²³. Increased proteases catabolize proteins whereas decreased amylase activity reduced hydrolysis of starch which is necessary for energy supply to developing embryos, this may be the reason for inhibition of seed germination at high concentrations of Copper. *Conocarpus lancifolius* did not show an efficient improvement on the rate of germination of *Cicer arietinum* under copper stress and found to be 30, 33, 35 and 42% at all supplied levels of copper, which may attribute to the oxidation of phenolic compounds present in it²⁴. Copper is an essential micronutrient required in a small amount and has a high affinity for solid phase organic matter, and may be accumulated on the surface of *Conocarpus lancifolius*^{25,7}.

Table 1: Effect of Cu and its biosorption by *Conocarpus lancifolius* on the rate of germination (%) of *Cicer arietinum* L.

S #	Cu (ppm)	Control plants	Experimental plants	Treated plants
1	0	40	-	-
2	25	-	44	30
3	50	-	45	33
4	75	-	38	35
5	100	-	35	42

Shoot length

Data presented in Table 2 showed drastic effects of copper on shoot length of *Cicer arietinum* and reduction was recorded up to 8.87 ± 1.99 , 9.33 ± 2.46 , 7.83 ± 2.88 , 8.31 ± 2.78 respectively at all applied concentration of copper, whereas it was 9.63 ± 0.80 cm in control. Shortening in shoot length due to toxic metal copper was also found by many previous workers²⁶⁻²⁸ may attribute to the affinity of heavy metal copper for biomolecules like carboxyl, sulfhydryl, phosphate, amide and amine groups that may cause cell wall loosening and in turn inhibition in mitosis²⁹. Reduction in shoot length may also be attributed to the poor root system because toxic metals first enter root tissues, persist there and cause drastic effects^{20, 7} and thus overall plant growth may be affected. Significant improvement was carried out by the application of *Conocarpus lancifolius* leaves in shoot length of *Cicer arietinum* plants up to 9.73 ± 0.68 , 10.66 ± 0.75 , 11.67 ± 1.52 and 10.83 ± 1.52 cm. Functional groups like ketone group, carboxylate groups, Hydroxyl group, Hydrocarbon, Amines, and Carbon oxygen group³⁰ present in *Conocarpus lancifolius* is negatively charged and behaves as an active ligand for positively charged salt ions like copper. According to the present study, *Conocarpus lancifolius* was found to be an effective biosorbent and an efficient fertilizer for the growth of plants³¹⁻³³.

Root length:

Data of present study revealed drastic effects of copper in root length of *Cicer arietinum* up to 25.27 ± 8.5 , 18.9 ± 1.2 , 23.7 ± 3.2 , 19.7 ± 2.5 cm respectively whereas in control it was 25.5 ± 7.15 cm. Copper at 25ppm does not show toxic effects on root length of *Cicer arietinum*² may attribute to the beneficial concentration as it is essential micronutrient for the plant. According to literature surveyed heavy metals have great affinity for proteins and lipids and plasma membrane which is composed of proteins and phospholipids is the first which combat with toxic metal copper³⁴ and increased potassium efflux by high concentration of Cu from roots of various plants such as wheat was noticed earlier³⁵ from where disturbance started and the growth was badly affected. Disturbance in

process of mitosis due to the high concentration of Copper may also be the reason of reduction in root length of *C. arietinum* at all applied levels of copper (Table 2). It is being observed that healthy roots play a key role in the virtuous growth of plants, but in a contaminated environment usually roots are first which face contaminations and try to retain contaminants like heavy metals and preventing them to reach in the shoot and any flaw or deformity due to Cu toxicity in roots causes stunted growth of plant²⁰. Significant improvement was exhibited by the application of *C. lancifolius* in root length of *C. arietinum* up to 28.8 ± 1.18 , 30.23 ± 3.81 , 29.01 ± 10.93 and 37.56 ± 3.78 cm over control (Table 2). *C. lancifolius* not only biosorbed the copper efficiently and improved root elongation significantly but it also provided several macro and micronutrients to the plants that are why root length increased as compared to control.

Leaf area:

Leaf area of *Cicer arietinum* growing under copper amended soil was found to be 0.26 ± 0.07 , 0.20 ± 0.06 , 0.15 ± 0.04 , 0.23 ± 0.23 respectively at all applied concentrations, whereas it was 0.27 ± 0.28 cm² in control plants (Table 2). Adverse effects of heavy metal copper exhibited on reduced leaf area of *C. arietinum* may attribute to the damaged root system (Table 2) or may be due to injured plasma membrane which caused inhibition in the conduction of water and mineral ions, leaf area reduced to overcome these stresses and to regulate a rate of transpiration. High concentrations of toxic metal Copper destroyed the mitochondrial structure in the plants and so their energy-producing activities were damaged³⁶. *C. lancifolius* supplied plants showed a significant improvement in the leaf area of *C. arietinum* up to 0.30 ± 0.03 , 0.38 ± 0.04 , 0.35 ± 2.09 , 0.26 ± 0.03 respectively at all applied levels of toxic metal copper. According to the data presented in Table 2, *C. lancifolius* efficiently biosorbed toxic metal copper and effectively enhanced leaf area by providing macronutrients and micronutrients present in them.

Table 2: Effect of *C. lancifolius* on shoot length, root length and leaf area of *C. arietinum* L. growing under copper stress.

S.#	Cu[ppm]	SHOOT LENGTH (cm)		ROOT LENGTH (cm)		LEAF AREA (cm ²)	
		Experimental	Treated	Experimental	Treated	Experimental	Treated
1	0	9.63±0.80	9.63±0.80	25.5±7.15	25.5±7.15	0.27±0.28	0.27±0.28
2	25	8.87±1.99	9.73±0.68	25.27±8.5	28.8±1.18	0.26±0.07	0.30±0.03
3	50	9.33±2.46	10.66±0.75	18.9±1.2	30.23±3.81	0.20±0.06	0.38±0.04
4	75	7.83±2.88	11.67±1.52	23.7±3.2	29.01±10.9	0.15±0.04	0.35±2.09
5	100	8.31±2.78	10.83±1.52	19.7±2.5	37.56±3.78	0.23±0.23	0.26±0.03

Results expressed as Mean ± SEM of triplicate.

Biomass:

Drastic effects of copper were found on biomass of *Cicer arietinum* up to 0.48±0.39, 0.66±0.12, 0.76±0.114, 0.69±0.26 gm respectively at all applied concentrations of copper whereas it was 0.85±0.203 gm in control. Literature surveyed reveals the adverse effects of varying concentrations of copper in various plants like *Cicer arietinum* and wheat³⁷. Salt enters plant with water through osmosis where it accumulates and stops various physiological functions by replacing some essential ions from enzymes and proteins³⁸ reduction in different growth parameters including root length, shoot length, leaf area and biomass may attribute to the osmotic injury and specific ions toxicity caused by copper³⁹⁻⁴¹. Reduction in biomass of *Cicer arietinum* growing under all applied concentrations of copper supports the data presented in Table 2, because the reduction in root length may cause inhibition in the conduction of water and minerals whereas inhibition in leaf area may cause deleterious effects on photosynthesis, which somehow or the other cause decline in biomass of *Cicer arietinum*. Application of magical powder of *Conocarpus lancifolius* leaves showed considerable improvement in biomass of *Cicer arietinum* up to 0.49±0.35, 0.64±0.11, 0.79±0.11 and 0.8±0.27 gm at all applied levels of copper. *Conocarpus* specie provided macromolecules like carbohydrates, lipids, and proteins which somehow or the other make complex molecules or ligands with copper and cleaned the plant

environment. *Conocarpus lancifolius* leaves not only biosorb copper but also provided macro and micronutrients⁵⁴ to *C. arietinum* which enhanced plants overall growth.

Relative water content (R.W.C):

Effect of Copper on the relative water content of *Cicer arietinum* mentioned in Table 2 was deadly and exhibited a decrease up to 39.4±5.35, 35.5±8.21, 11.83±9.54 and 20.5±18.5 % respectively at all applied levels of copper whereas it was 41.67±2.9 % in control.

Inhibition in the relative water content of *Cicer arietinum* confirmed the results presented in Table 1 because conduction of water reduced due to the damaged root system and hence decreased relative water content which ultimately declined leaf area just to regulate the rate of transpiration⁴². Relative water content is a stress indicator which exhibited the status of plant growth regarding root/shoot length, leaf area, biomass and other physiological functions like photosynthesis. Literature surveyed revealed that under heavy metal stress the water transport in plant reduced and resulted in water deficit in the plants^{43,44}. Application of *Conocarpus lancifolius* significantly improved the relative water content of *Cicer arietinum* up to 43.6±4.7, 47.76±13.5, 36.67±18.5 and 31.6±4.14 % at all supplied concentrations of copper Table 2. Biosorption of copper by *Conocarpus* depends on the relative amount of toxic metal ions and biosorbent³⁰.

Table 3: Effect of Copper and its biosorption by *C. lancifolius* on biomass (gm) and relative water contents (%) of *Cicer arietinum*.

S. No.	Cu [ppm]	BIOMASS (gm)		R.W.C (%)	
		Experimental	Treated	Experimental	Treated
1	0	0.85±0.203	0.85±0.203	41.67±2.9	41.67±2.9
2	25	0.48±0.39	0.49±0.35	39.4±5.35	43.6±4.7
3	50	0.66±0.12	0.64±0.11	35.5±8.21	47.76±13.5
4	75	0.76±0.114	0.79±0.11	11.83±9.54	36.67±18.5
5	100	0.69±0.26	0.78±0.27	20.5±18.5	31.6±4.14

Results expressed as Mean ± SEM of triplicate.

Photosynthetic pigments

Chlorophyll ‘A’

Current data revealed that toxicity of copper imposed a deleterious effect on chlorophyll a of *Cicer arietinum*. Reduction in chlorophyll an of *Cicer arietinum* was found to be 0.725±0.06, 0.647±0.10, 0.784±0.02, 0.7159±0.07 at all supplied concentrations of copper respectively, whereas it was 0.8726±0.43 mg/gm F.wt in control leaves (Table 4). Reduction in chlorophyll a may attribute to the decline in volume and number of Mesophyll chloroplasts of *Cicer arietinum* leaves growing under Cu stress²⁰. Several reports showed chlorophyll biosynthesis inhibition by metals in higher plants⁴⁵⁻⁴⁸. Heavy metal Copper replaced magnesium of porphyrin ring of chlorophyll due to which leaf remain green but nonfunctional hence inhibited process of photosynthesis^{45,49}. A significant improvement in chlorophyll a of *Cicer arietinum* leaves was noted in *Conocarpus lancifolius* supplied plants up to 0.738±0.02, 0.846±0.10, 0.798±0.01 and 0.7488±0.10 mg/gm F.wt, this may attribute to the ligand forming capability of macromolecules of *Conocarpus lancifolius* which is a salt-tolerant species⁵⁰⁻⁵² and contribution of its micronutrients as effective fertilizer to the *Cicer arietinum*. Moreover Copper has a high affinity for the solid phase organic matter, which can accumulate on the surface of *Conocarpus lancifolius*^{7,25}.

Chlorophyll ‘B’

Deleterious effects of Copper were found on chlorophyll ‘b’ of *Cicer arietinum* up to 0.1942±0.003, 0.1557±0.002, 0.323±0.002 and 0.292±0.01 mg/gm f.wt at all applied

concentrations of copper respectively, whereas it was 0.245±0.01 mg/gm f.wt. in control plants (Table 4). High concentration of copper may deform chloroplast by replacing Mg and consequently lead to disruption of photosynthetic machinery Table 3^{20,45,48,49}. Cu imposed adverse effects on *Cicer arietinum* may attribute to the redox behavior of the metal, which leads to the exacerbate formation of reactive oxygen species, inducing to severe damage such as biological membrane degradation and protein denaturation⁷.

Conocarpus lancifolius supplied plants showed noteworthy improvement in *Cicer arietinum* up to 0.282±0.009, 0.315±0.001, 0.279±0.002 and 0.27±0.01 mg/gm f.wt may attribute to the high efficiency of metal binding to free sites present in *Conocarpus lancifolius*.

Total chlorophyll

Adverse effects of copper were found on total chlorophyll up to 0.918±0.06, 1.02±0.01, 0.803±0.35 and 1.08±0.05 in *Cicer arietinum* leave growing under applied levels of copper respectively. Whereas it was 1.11825±0.01 mg/gm f.wt in control (Table 4). Copper is an important micronutrient that regulates vital physiological functions of plants as a catalyzer of redox reaction in mitochondria, chloroplasts, and cytoplasm of cells⁵³ or as an electron carrier during plant respiration⁵⁴. However, at higher concentrations, Copper becomes lethal to plant tissues⁴⁰ and inhibit drastically all physiological functions as it has a great affinity for proteins, enzymes and porphyrin ring of chlorophyll⁵⁵. Cu was shown to inhibit growth and to interfere with important cellular processes such as photosynthesis and respiration⁵⁶. The present study

showed an improvement in total chlorophyll contents of *Conocarpus lancifolius* treated plants up to 1.02±0.01, 1.162±0.01, 1.069±0.0 and 1.019±0.015 at all supplied levels of copper. The data suggested that *Conocarpus lancifolius* is provided with an efficient mechanism against stress which is not only low cost, easily and abundantly available but also eco-friendly biosorbent.

Application of *C. lancifolius* significantly detoxified *Cicer arietinum* by toxic copper as 19.18±0.11, 38.62±16.3, 71.67±2.2 and 24.01±28.84 mg/gm f.wt. *Conocarpus lancifolius* efficiently detoxify the effects of copper and enhanced synthesis of glucose via photosynthesis⁵⁸, (Table 4 & 5).

Table 4: Effect of *C. lancifolius* on photosynthetic pigments of *C. arietinum* growing under copper stress (mg/gm F.wt).

Cu[ppm]	Chlorophyll a		Chlorophyll b		Total chlorophyll		Carotenoid	
	Experimental	Treated	Experimental	Treated	Experimental	Treated	Experimental	Treated
0	0.873±0.43	0.873±0.43	0.245±0.01	0.245±0.01	1.118±0.01	1.118±0.01	0.605±0.001	0.605±0.001
25	0.725±0.06	0.738±0.02	0.194±0.003	0.282±0.009	0.918±0.06	1.02±0.01	0.47±0.01	0.629±1.19
50	0.647±0.10	0.846±0.10	0.1557±0.002	0.315±0.001	1.02±0.01	1.162±0.01	0.422±0.01	1.191±0.025
75	0.784±0.02	0.798±0.01	0.323±0.002	0.279±0.002	0.803±0.35	1.069±0.02	0.56±0.02	0.667±0.02
100	0.7159±0.07	0.7488±0.10	0.292±0.01	0.27±0.01	1.08±0.05	1.019±0.015	0.563±0.01	0.659±0.025

Results expressed as Mean ± SEM of triplicate.

Photosynthetic products

Glucose:

Current data revealed drastic reductions in glucose contents of *Cicer arietinum* growing in copper amended soil and found to be 14.28±4.3, 29±36.13, 26.8±13.37 and 8.38±4.74 at all applied levels of copper respectively. Glucose content of control was found to be 16.64±13.55 mg/gm f.wt (Table 5). Copper as a micronutrient involved in various physiological functions of the plant as a catalyzer⁵³ or as an electron carrier⁵⁴, but present data showed a reduction in glucose even at 25ppm copper, which proved the results of Table 3, where chlorophyll a & b and total chlorophyll reduced. Reduction in glucose may attribute to the polymerization of glucose to sucrose (Table 4). At higher concentrations, copper became toxic and replaced magnesium from porphyrin ring of chlorophyll, made it non-functional and distorted and hence chlorophyll reduced (Table 3). Due to destroyed photosynthesis (in order to produce glucose for glycolysis) polymerized sucrose molecules broke down which ultimately increased glucose and decreased sucrose molecules (50 & 75ppm)^{45,54,55,57}. Glucose and sucrose were badly affected at 100ppm copper in *Cicer arietinum*.

Sucrose:

Current data revealed the deleterious effects of copper on Sucrose of *Cicer arietinum* to be 149.6±2.3, 94.4±1.4, 70.42±10.1 and 40.93±50.6 whereas it was 71.14±5.28 mg/gm f.wt. in control. Reduction in chlorophyll (Table 3) induced a decrease in photosynthesis hence created stress in the plant body. Higher sucrose accumulation in damaged leaves may result both in the higher resistance of their photosynthetic apparatus⁵⁹. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive center of ribulose biphosphate carboxylase⁶⁰. Inhibition in sucrose at higher concentrations (75 & 100ppm) indicates that Copper acts as negative biocatalyst and inhibits or slows down the processes of polymerization⁵⁷.

Application of *Conocarpus lancifolius* showed the enhanced effect on sucrose of *Cicer arietinum* up to 90.43±33.08, 67.1±14.5, 105.96±33.7 and 42.27±11.5. The *Conocarpus lancifolius* not only efficiently biosorbed copper from *Cicer arietinum* but also provided its macro and micronutrients for better plant growth.

Table 5: Effect of *C. lancifolius* on photosynthetic products of *Cicer arietinum* growing under copper stress.

S.#	Cu[ppm]	GLUCOSE		SUCROSE		TOTAL SOLUBLE SUGAR	
		Experimental	Treated	Experimental	Treated	Experimental	Treated
1	0	16.64±13.55	16.64±13	71.14±5.3	71.14±5.3	117.43±16.9	117.43±16.9
2	25	14.28±4.3	19.18±0.1	149.6±2.3	90.43±33	62.73±1.17	91.88±34.6
3	50	29±36.13	38.62±16	94.4±1.4	67.1±14.5	100.2±3.305	134.13±1.19
4	75	26.8±13.37	71.67±2.2	70.42±10.1	106±33	82.43±2.28	108±3.72
5	100	8.38±4.74	24.01±29	40.93±50.6	42.27±12	97.53±3.69	103.5±22.39

Results expressed as Mean ± SEM of triplicate.

Total soluble sugars:

Sugars considered as the main source of energy to operate all physiological functions of the plant. Current data revealed the drastic effects of copper on total soluble sugars of *Cicer arietinum* up to 62.73±1.17, 100.2±3.305, 82.43±2.28, and 97.53±3.69 whereas it was 117.43±16.9 mg/gm f.wt., in control (Table 4). A vital role of soluble sugars (reducing and non reducing) including sucrose, glucose, and fructose, is evident in plant structure and metabolism. In addition to the role of sugars in osmoregulation, the soluble sugars allow the plants to maximize sufficient carbohydrates storage reserves to support basal metabolism under the stressed environment⁶¹. However high light irradiance, heavy metals, nutrient shortage and ozone decreased soluble sugar concentrations⁶². Deleterious effects of copper on total soluble sugars of *Cicer arietinum* may attribute to the reduction in the number of chlorophyll contents (Table 3). Application of *Conocarpus lancifolius* significantly biosorbed copper and enhanced total soluble sugars in *Cicer arietinum* up to 91.88±34.6, 134.13±1.19, 108±3.72, 103.5±22.39 mg/gm f.wt⁶³. *Conocarpus lancifolius* are negatively charged and behaves as an active ligand for positively charged salt ions like copper³⁰.

CONCLUSIONS

As heavy metal contamination has become a universal threat to life, these toxic metals have to be eliminated from the soil through some procedure which should be cost-effective and eco-friendly. Current research work was designed to investigate the efficiency of *Conocarpus lancifolius* for the biosorption of copper from *Cicer*

arietinum. Results of the present study showed that biosorbent *C. lancefolius* not only actively biosorbed heavy metal copper but also provide macro and micronutrients and enhanced plant's growth. *C. lancefolius* was found an efficient, easily available and eco-friendly biosorbent which should be investigated for other important crops for biosorption of other heavy metals. Apart from this, laborious literature survey revealed that *Conocarpus lancifolius* is an antifungal and antibacterial plant and may use in reducing bacterial and fungal contaminations.

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