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THE EFFECT OF $MgSO_4$ ON BEHAVIOR OF THE PATHOGENIC FUNGUS, *FUSARIUM SOLANI* AND THE RATE OF SEEDLINGS DAMPING OFF DISEASE ON SESAME

Jamal H. Kadhim,Akeel E. Mohammed, SalwanAbdAlzahraJabbarAllwbawi and Akram A. Mohammed

Department of Plant Protection, Faculty of Agriculture, University of Kufa, Najaf, Iraq.

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ABSTRACT : This study was conducted to determine the effect of adding $MgSO_4$ on *Fusariumsolani* that infect sesame seedlings and plant growthin both in vitro and greenhouse conditions. $MgSO_4$was significantly increasedthe activated units (spores) of *F. solani* growing in Petri plates of sameseedling damping off when *F.solani* was grown on Petriplates, wherethere was a positive relation between the increasing of $MgSO_4$ concentrationandnumber of spore growing of *F.solani*under laboratory conditions. Glasshouse results showed that the percentage ofsesame seedlings infected with *F. solani* was significantly increased with increasing the concentration of $MgSO_4$and all treatments were significantly higher compared tocontrol treatment. The results were also showed thatleaves contain of Auxin and Gibberellin hormones were increased when $MgSO_4$ concentration increased either the soil was contaminated with *F.solani*spores or not. However, the amount of these hormones was higher when no fungus added to the soil.Abscisic acid of sesame leaves was not affected with increasing the concentration of MgSo4 either abundance or absent of *F. solani* in the soil.

Key words : *Fusarium solani*, $MgSO_4$, auxin, gibberellin, abscisic acid.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of oil crops that used in many food industries due to its contains of oil, proteins, carbohydrates, and some elements such as calcium and phosphorus. In addition, the peel of sesame seeds is rich in fibers and vitamin E that has medical uses against some human diseases (Namik, 2007). There are many diverse and significant fungal diseases infect sesame and cause serious yield loss. One of these fungal diseases is sesame seedling damping off that causes by *Fusariumsolani* (Mart.) App. et. Wr. emend. Snyder & Hansen) (Agrios, 2005). *Fusarium solani* is widespread fungal pathogen that causes many plant diseases (Agrios, 2005). The sexual stage of *F. solani* (*Nectriahaematococca* Berk. & Broome) belongs to Ascomycetes (Leslie and Summerell, 2006). The fungus has the ability to produce many types of enzymes and mycotoxins (Nelson *et al*, 1997). *F. solani*has been isolated from different environmental areas and infect wide range of plant hosts (Leslie and Summerell, 2006). Agrios, (2005) mentioned that *F. solani* and *Rhizoctoniasolani*were the main fungal speciesthatcause seedlings damping off, while, (Ammar, *et al*, 2004, Silme and Çagirgan, 2010) reported that *F. solani* was infected all growth stages of sesame plant. *Fusariumsolani* attacks sesame seeds before germination leading to seed decay and also infect seedlings before emergence causing pre–emergence damping–off or after emergence causing post–emergence damping–off (Bilgrami and Verma, 1981, Gray and Achenbach, 1996).

Middle East countries particularly Iraq are suffering from the drought and the shortage of fresh water which might be continued in further (FAO, 2012). This challenge has provoked a search to find new strategies to deal with same circumstances such as using saline water to irrigate crops. Many reports have mentioned that there is a potential of using saline water by studying the electrical conductivity in plants (Challa and VanBeusichem, 2004, Jamil *et al*, 2005, Munns, 2005, Eraslan *et al*, 2008) or determining the effect of each salt of saline water individually in plants (Cicek and Cakirlar, 2002, Kaydan *et al*, 2007, Afzal and Asghari, 2008). However, published research papers onthe effect of salinity water on growing and behaviour of plant fungal pathogens are not available. Thus, the main objective ofthe current researchwas to study the effect of $MgSO_4$ on *F. solani* the causal agent of seedling damping off disease on sesame.

MATERIALS AND METHODS

*F. solani*isolates and their pathogenicity

All *F. solani*isolates used in this study were supplied by laboratory of Fungi - Plant Protection Department – Faculty of Agriculture – University of Kufa. Isolates were re-cultured on9-cm sterile Petri dishes containingpotato dextrose agar (PDA)thenits pathogenicity was tested by inoculated the centre of another Petri plates contain PDA with 0.5cm disk of *F. solani* hyphae and incubated for 48h. Sesame seeds were sterilized by 2% sodium hypochlorite solution for 3min then washed with sterilized distilled water for several times and planted on edges of the fungus colonies. Four replicates each with twenty seeds were contaminated with *F. solani*and another four replicates each with twentyseeds were sprayed withsterilized distilled wateras a control. Then, all replicates were incubated at 25± 2°C for 10 days. Afterward, the number of rotting seeds and infected seedlings were calculated.

Determining the $MgSO_4$ concentration in saline water of Najaf Province

Samples of saline water were collected from two towns of the Najaf city that includeAlhuria and Alabbasia. All samples were mixed together and then Mg, SO_4 were estimated as follows:

The estimation of Mg concentration

Titration method was used to estimate Mg concentration (Page *et al*, 1981).

The estimation of SO_4 concentration

To determine the SO_4 concentration, 5ml of saline water was added to 25ml watery di-calcium chloride in a 100 glass flask andshaken for 30 min using electrical shaker (180 c/min). The suspension was filtered using Whatman No. 1 filter papers to obtain colourless extract. Then, 10ml of this extract was added to10ml of $Ca(OCl)_2$ using 50ml conical centrifuge tubes. Optical absorption was recorded for tested samples and standard's solutions directly on 470 nanometre. 1ml of HCL was added then records were applied to standard curve (Page *et al*, 1981).

Total volume of the extract (ml)

$$\text{Sulphate}\,(\text{mg.L}^{-1}) = \frac{\text{Sulphate (from standard curve)}}{\text{The weight of air dry soil (g)}}$$

The effect of $MgSO_4$ concentration on circle growth area of *F. solani* in Petri dishes

$MgSO_4$ concentration was measured in the collected samples as an average 0.005 $g.L^{-1}$. Synthetic concentrations of $MgSO_4$ were made by melting 0.005gm in PDA then divided in three 250cm^3 glassflasks; the flasks were shaken to get $MgSO_4$ melted.Another PDA was provided without $MgSO_4$ as a control, all flasks were autoclaved in 121°C and 15 pound/$inch^2$pressure for 20 min. sterilized media were placed in 9cm Petri plates and the centre of the all plates was inoculated with 0.5cm disk of *F. solani* hyphae. Four replicates of each $MgSO_4$concentration were incubated at 25± 2°C. When the growth of *F. solani* colonies was reached 4.5cm, the diameter of each colony was recorded as follows:

Growth area of *F. solani*= (the diameter of fungus colony in 9cmPetri plates)^2 *(3.14)

The effect of $MgSO_4$ concentration on spores density of*F. solani* in Petri plates

The procedure described byFawrou*et al*, (2014) was used to prepare the spores suspension. One Petri plate contains 7 days colony of *F. solani* was randomly taken and it contains were placed in a beaker with 150mlsterilized distilled water and 0.02% of tween 80 then mixed by electrical mixture on 150/min for two hours, the mixture was filtered. 1ml of 10^{-6}suspensionwas taken

Table 1 : The effect of *F. solani* on the percentage of sesame seeds damping off on Petri plates after 10 days of planting.

F. solani	% percentage	
	seeds damping off	seedlings damping off
With *F. solani*	8.125	30.00
Without *F. solani*	0.00	0.00
L.S.D=0.05	2.480	2.997

Table 2 : The effect of $MgSO_4$ concentration on circle growth area of *F. solani* in Petri plates

$MgSO_4$ concentrations ($g.L^{-1}$)	Circle growth area of *F. solani* (cm^2)
0.00 (DI water)	38.32
0.003	43.75
0.005	54.08
0.007	63.58

Table 3 : The effect of MgSo4 concentration on spores density of *F. solani* *10^6

$MgSO_4$ concentrations $g.L^{-1}$	Spores density of *F. solani* *10^6
0.00 (DI water)	13.66
0.003	22.63
0.005	38.24
0.007	50.97
L.S.D = 0.05	2.365

by pipette and the spores density was calculate using Hemocytometer following Jason Fan method(2016).

The effect of different $MgSO_4$ concentrations on the percentage of sesame damping off in Petri plates

Solid media with and without MgSO4 concentrationswere provided and inoculated with *F. solani*. Twenty sesame seeds were placed on edges of the fungus colonies. Four replicates each with twenty seeds were contaminated with *F. solani*and another four replicates each with twenty seeds were sprayed with sterilized distilled water as a control. Then, all replicates were incubated at 25± 2ºC for 10 days. Afterward, the number ofrotting seeds and infected seedlings were calculated.

The effect of different $MgSO_4$ concentrations on somegrowth characteristicsof *F. solani*and theireffect on sesame plants grown in pots.

Table 4 : The effect of different $MgSO_4$ concentrations on the percentage of sesame seeds damping off in Petri plates.

$MgSO_4$ concentrations ($g.L^{-1}$)	% Percentage of seeds damping off	
	With *F.solani*	Without *F.solani*
0.00 (DI water)	8.125	0.00
0.003	16.000	0.00
0.005	24.812	0.00
0.007	37.250	0.00
L.S.D = 0.05	1.139	

Table 5 : The effect of different $MgSO_4$ concentrations on the percentage of sesame seedlings damping off disease in Petri plates

$MgSO_4$ concentrations ($g.L^{-1}$)	% Percentage of seedlings damping off	
	With *F.solani*	Without *F.solani*
0.00 (DI water)	30.00	0.00
0.003	43.750	0.00
0.005	51.250	0.00
0.007	62.750	0.00
L.S.D = 0.05	2.230	

Table 6 : The effect of watering by different $MgSO_4$ concentrations on the percentage of sesame seeds damping off in greenhouse after 10 days of planting.

$MgSO_4$ concentrations ($g.L^{-1}$)	% Percentage of seeds damping off	
	With *F.solani*	Without *F.solani*
0.00 (DI water)	6.250	0.00
0.003	7.583	0.00
0.005	11.666	0.00
0.007	16.583	0.00
L.S.D = 0.05	5.253	

Table 7 : The effect of watering by different $MgSO_4$ concentrations on the percentage of sesame dead plants in greenhouse after 28 days of planting.

$MgSO_4$ concentrations ($g.L^{-1}$)	% Percentage of sesame dead plants	
	With *F.solani*	Without *F.solani*
0.00 (DI water)	20.00	0.00
0.003	28.75	0.00
0.005	35.00	0.00
0.007	43.75	0.00
L.S.D = 0.05	4.768	

Soil samples were grinded, sterilized and dried then divided into two groups in 30 x 24 pots (2kg/pot). The first group was contaminated with 10^6 *13.66 of *F. solani* /gm soil and mixed well and the second group was not contaminated. Twentysesameseedswere planted in each pot and covered by a layer of soil. 0.003, 0.005 or 0.007 concentrationsof $MgSO_4$ were melt individually in one litre of sterilized water. Four pots were contaminated with *F. solani*and another four replicates were sprayed with sterilized distilled water as a controlfor each concentration used.Pots were placed in the net house andwatered by $MgSO_4$ solutions (control treatment watered with water only). The percentage of rotting seeds and infected seedlings were recorded after 10 days. The wilted plants and plant growth regulators were recorded after 28 days of planting.

The estimation of leaves sesame contains of Auxin, Gibberellin and Abscisic acid

Auxin, Gibberellin and Abscisic acid hormones were estimated following the procedure of Eraslan*et al*, (2008) were 1g samples of fresh leaves were added to 12ml methanol, 5ml chloroform and 3ml ammonium hydroxidethen the volume was completed to 25ml by sterilized distilled water.15ml of ethyl acetate was added and the mixture was steamed for one hour and the acidity was controlled by drops of HCL and sodium hydroxide. Samples estimated using UV-visible Spectrophotometer on wavelength 222 nanometre forAuxin, 254 nanometre for Gibberellin. The method of Srivastava and Prased (2010) was used to extract Abscisic acid using High Performance Liquid Chromatography (HPLC) on 265 nanometre.

Data analysis

Statistical analyses were conducted using GenStat (version 12; VSN International, Hemel Hempstead, UK). Normality of data distribution was estimatedusing a Shapiro–Wilk test (W test). The data weretransformed

using arcsine square root when it wasnecessary to meet the assumption of normality. Mean comparisons were performed usingLSD test at 5% level of significance ($P < 0.05$).

RESULTS AND DISCUSSION

The effect of *F. solani* on the percentage of sesame seeds damping off on Petri plates

Table (1) showed that *F. solani* was significantly increased the percentage of sesame seeds damping off on Petri plates compared with 0.00% at control treatment.

The effect of $MgSO_4$ concentration on circle growth area of *F. solani* in petri plates

Results of Table (2) showed that all concentrations of $MgSO_4$ (0.003, 0.005 and 0.007) were increased the growth of *F. Solani*in comparison with control. In addition, concentration of $MgSO_4$ had a significant effect on the circle growth area of *F. solani* colonies where it was significantly increased when the concentration of $MgSO_4$ was increased.

The effect of $MgSO_4$ concentration on spores density of *F. solani* $*10^6$

Spores density of *F. solani* was significantly increased $(22.63, 38.24 \text{ and } 50.97)*10^6$ with increasing of $MgSO_4$concentration.

The effect of different $MgSO_4$ concentrations on the percentage of sesame seeds damping off in Petri plates

There was significant increasing of sesame seedling damping off when *F.solani* was grown on Petri dishes, while the increasing of $MgSO_4$ concentration increased the growth and spores of *F.solani* in laboratory. Glasshouse results showed that the percentage of infected seedlings by the pathogen with different concentration of $MgSO_4$ was increased in comparison with control treatment.

The effect of different $MgSO_4$ concentrations on the percentage of sesame seedlings damping off in Petri plates

Different $MgSO_4$ concentrations (0.003, 0.005, and 0.007) affect the percentage of sesame seedlings damping off disease in Petri plates (30.00, 43.750, 51.250, and 62.750) respectively in comparison with the control.

The effect of irrigating by different $MgSO_4$ concentrations on the percentage of sesame seeds damping off in greenhouse after 10 days of planting

Irrigating of pots contain sesame seeds by different $MgSO_4$ concentrations showed significant effect on *F. solani*precipitate the exploitation of sugars which increase the number of produced spores. The percentage of infected seeds was (0.00) in pots with no fungus treatments. While pots with *F. solani*and irrigated with0.003, 0.005, and 0.007 of $MgSO_4$showed sesame seeds damping off 7.538, 11.666 and 16.583%respectively.The percentage of sesame infected seeds was higher when $MgSO_4$ concentration increased.

The effect of irrigating by different $MgSO_4$ concentrations on the percentage of sesame dead plants in greenhouse after 28 days of planting

There were no infected seedlings in thepots with no fungus after 28 days of planting. While the percentage of sesame dead seedlings in pots with *F. solani*were 28.75, 35.00 and 43.75% respectively after irrigating pots with either 0.003, 0.005, and 0.007 of $MgSO_4$. The percentage of sesame infected seedlings was higher when $MgSO_4$ concentration increased.

Table 8 : The effect of watering by different $MgSO_4$ concentrations on sesame leafs contains of Gibberellinwithin or without *F. solani*after 28 days of planting

$MgSO_4$ concentrations ($g.L^{-1}$)	The amount of Gibberellinon sesame leafs After 28 days of planting	
	With *F.solani*	Without *F.solani*
0.00 (DI water)	0.222	0.228
0.003	0.253	0.269
0.005	0.277	0.326
0.007	0.300	0.368
L.S.D = 0.05	0.058	

Table 9 : The effect of watering by different $MgSO_4$ concentrations on sesame leafs contains of Abscisic acid within or without *F. solani* after 28 days of planting

$MgSO_4$ concentrations ($g.L^{-1}$)	The amount of Abscisic acid on sesame leafs After 28 days of planting	
	With *F.solani*	Without *F.solani*
0.00 (DI water)	0.201	0.200
0.003	0.202	0.200
0.005	0.203	0.200
0.007	0.205	0.200
L.S.D = 0.05	0.0089	

Table 10 : The effect of watering by different $MgSO_4$ concentrations on sesame leafs contains of Auxin acid within or without *F. solani* after 28 days of planting

$MgSO_4$ concentrations ($g.L^{-1}$)	The amount of Auxin acid on sesame leafsAfter 28 days of planting	
	With *F.solani*	Without *F.solani*
0.00 (DI water)	0.179	0.185
0.003	0.201	0.218
0.005	0.220	0.235
0.007	0.241	0.260
L.S.D = 0.05	0.013	

The effect of watering by different $MgSO_4$ concentrations on sesame leafs contains of Gibberellin within or without *F. solani* after 28 days of planting

Glasshouse results showed that *F. solani* pathogenicity with different concentrations of $MgSO_4$ was increased in comparison with control treatment. The analysis of sesame leaves contains was shown that Gibberellin hormone concentrations were increased when $MgSO_4$ concentration increased either the pathogen was in soil or not. The amount of this hormone was higher when no fungus added to the soil Table (8).The Gibberellin was 0.269, 0.326 and 0.368respectively when watering by 0.003, 0.005 and 0.007 $MgSO_4$ concentration with no pathogen. While the hormone was 0.253, 0.277 and 0.300 when watering by mentioned $MgSO_4$ concentrations within *F. solani*.

The effect of watering by different $MgSO_4$ concentrations on sesame leafs contains of Abscisic acid within or without *F. solani* after 28 days of planting

Table (9) showed the effect of watering sesame pots with different concentrations of $MgSO_4$ and plant leafs contains of Abscisic acid after 28 days of planting. There were no significant effect for irrigate plants with $MgSO_4$ concentrations either with *F. solani* or without. Results of this study confirmed that the amount of Abscisic acid in leafs was higher when using high concentration of $MgSO_4$.

The effect of watering by different $MgSO_4$ concentrations on sesame leafs contains of Auxin acid within or without *F. solani* after 28 days of planting

Watering by different $MgSO_4$ concentrations was increased sesame leafs contains of Auxin acidand the amount of the hormone was higher when high $MgSO_4$ was used to irrigate plants. The amount of Auxin acid was higher (0.218, 0.235 and 0.260) when the soil of pots not contaminated with the pathogen. Whereas, contaminated the soil of pots with *F.solani* was decreased the hormone level in sesame leafs that amounted (0.201, 0.220 and 0.241) respectively for the three concentrations of $MgSO_4$. Results showed that using 0.007 concentration of $MgSO_4$ to irrigate plants without *F.solani* had significant on hormone level on sesame leafs.

$MgSO_4$was significantly increased the activated units (spores) of *F. solani* growing in Petri plates of sesame seedling damping off, where there was a positive relation between the increasing of $MgSO_4$ concentration and number of spore growing of *F.solani* under laboratory and glasshouse conditions.*F. solani* has the ability to infect sesame seeds and seedlings after 10 days of planting on Petri plates. This result is consistent with (Gray and Achenbach, 1996, Ammar, 2004, Agrios, 2005, Silme and Çagirgan, 2010), they mentioned that the pathogen has the ability to infect plants in all growth stages. The reason for sesame seeds and seedlings damping off might be due to mycotoxins that produced by *F. Solani* which can break the cell wall of the host. This is in agreement with (Nelson *et al*, 1997) who reported that *F. Solani* produces Chitinase, Protease and Cellulase and also some phytotoxins such as Fusaric acid, Anhydrofusarbin, Javanivine and polypeptidetoxin.

$MgSO_4$ was clearly enhanced the growth of *F. solani*on Petri plates. This might be because its contribution in some metabolism reactions of the fungus. Kaydan*et al*, (2007) mentioned that some cations and anions such as Mg and SO_4 were important to fungus growth. Bilgrami and Verma, (1981), Griffin, (1981), Landecker, (1982) reported that Sulphur was essential to fungi, all types of sulphur used by its association with organic sulphurous such as sulphurous amino acids (Cystin,Gleotoxin, Cysteine, and Methionine), vitamins (Thiamineand Biotin), enzymes of SH-Sulfhydyl group, proteins, Glutathione and antibiotics. Magnesium is also vital for fungi; it activates the enzymatic systems, cell membranes, cell walls, nucleic acids and ribosomes. Also, Magnesium plays an important role in ions inhibition (Sharif, 2012a).

Spores density of *F. solani* was significantly increased with increasing of $MgSO_4$ concentration.This may related to the contribution of sulphur in producing spores. Sulphur may enhance the fungus to produce vitamins that are essential to produce spores and precipitate the exploitation of sugars which increase the number of produced spores (Bilgrami and Verma, 1981).This finding was confirmed the results obtained by (Sharif, 2012b) who reported that using $MgSO_4$in growing media promote fungi to produce more spores.

The percentage of infected seeds, seedlings in Petri plates and pots after 10 and 28 days of planting was not affected when there was no *F. solani* added to treatments even when high concentration of $MgSO_4$ used. The growth of *F. solani* on PDA media that contains $MgSO_4$ or in pots that contaminated with the fungus and irrigated with MgSO4 solution was higher, and made significant increasing of seeds and seedlings damping off. Diseased seeds and seedling of sesame were increased when high concentration of $MgSO_4$ used either in PDA or irrigation. $MgSO_4$ leads to high density of pathogen spores which probably the cause of increasing fungus pathogenicity (Agrios, 2005). The results of current study that showed significant density of *F. solani* spores when $MgSO_4$concentration was increased Table (3), also the

growth area of the fungus was increased Table (2), the metabolism of the pathogen and producing of mycotoxins was increased. Nelson *et al*, (1997) mentioned previously that *F. solani* has the ability to produce enzymes such as chitinase, Protease and cellulase and phytotoxins (Fusaric acid, Anhydrofusarbin, Javanivine and polypeptidetoxin) that play significant role of fungus pathogenicity.

The increasing of $MgSO_4$ concentration in irrigation water without *F. solani* had no effect on the amount of Abscisic acid. This result promotes a previous study by Hermans*et al*, (2010). A little increasing of Abscisic acid was accrued when high concentrations of $MgSO_4$ were used to irrigate plants that contaminated with the pathogen. The effect of $MgSO_4$ was clearly showed in Table 1and 2 when the growth and spores number were increased. The disruption of root system in sesame plants may occur due to the increasing of fungus in the soil, as a result, plants produce more Abscisic acid as a defense mechanism (Bohnert and Jensen, 1996). Plants also use different mechanisms to start some physiological and chemicals changes (Bohnert and Jensen, 1996, Shinozaki and Yamaguchi Shinozaki, 1997). For instance, the increasing of Abscisic acid in leafs (Davies and Zhang, 1991) and another plant cells (Shinozaki and Yamaguchi Shinozaki, 1997).

The amount of Gibberellin and Auxin acid in sesame leafs was higher when the soil not contaminated with the pathogen, this may related to mycotoxins or enzymes that produce by the fungus (Afzal and Asghari, 2008) which affect the root system of plant and prevent the absorption of dissolved nutrients (Olsen, 1999). Mycotoxins can be absorbed by the roots and lead to physiological changes in plants (Hasegawa *et al*, 2000, Sharif, 2012a) or prevent absorption of some important elements to plants (Burns *et al*, 2013, Kadhim and Dewan, 2014). All these unusual changes may affect the vital bio-interactions of plant. Current study shows that using high concentration of $MgSO_4$ in irrigation water either the soil contaminated with *F.solani* or not promoted the producing of Gibberellin and Auxin acid in sesame leafs. This may because $MgSO_4$ made perfect conditions for plants to grow (Taiz and Zeiger, 2002) as it contains magnesium which plays unique roles in plant physiology. Magnesium is the centre of molecule chlorophyll and it affects the enzyme system particularly the metabolism of carbohydrates. Also magnesium is tonic for many enzymes such as phosphorylases and carboxylases and it considers as a link between the enzyme and its subject (Cakmak and Kirkby, 2008, Cakmak and Yazici, 2010, Ibrahim, 2010, Srivastava, 2010, Cakmak, 2013, Gransee and Führs, 2013, Huber and Jones, 2013, Mengutay*et al*, 2013). In addition to phosphor metabolism as the magnesium is important to enzymes that transfer phosphates (Verma, 2008).

Sulfur as a part of $MgSO_4$ is important element to plants which has significant role in balanced fertilization that lead to high productivity. It performs many physiological functions such as create amino acids (Methionine and Cysteine) proteins and sulfolipds. Sulfur is also forms many important bio-compounds (Glutathione, Biotine, Thiamine, Coenzyme A and Glycosides), 3D proteins and sulphur bonds (S-S) (Verma, 2008, Sharif 2012a).

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