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Bio-efficacy of *Pseudomonas fluorescens* (7% WP and 5% SC formulations) against bacterial wilt disease of chilli

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***Abstract*—** Bacterial wilt caused by *Ralstonia solanacearum* is an important disease of chili crop in West Bengal. *P. fluorescens* was employed to manage *R. solanecearum* under field condition. The combined use of seed and soil treatment were most effective (0.83 to 10.82% PDI) than sole use of seed or soil treatment (3.33 to 24.98% PDI). Vegetative growth and yield of chili were also influenced through integration of seed and soil treatments with *P. fluorescens* 7% WP @ 10 g/kg seed + 2.5 kg/ha as well as with *P. fluorescens* 5% SC applied @ 10 ml/kg seed + 2.5 lit/ha . The seed treatment when followed by soil treatment showed best result in respect of fresh root weight (6.63-7.43 g), dry root weight (2.53-2.80 g), fresh shoot weight (55.45-60.23 g), dry shoot weight (24.25-26.33 g), plant height (44.83-47.53 cm) and yield (257.18-265.60 kg/ha) than control. There was no adverse effect of *P. fluorescens* 7% WP and *P. fluorescens* 5% SC on soil beneficial microbes. The products increased the root and shoot weight, dry weight of seedlings and seedling vigor index than control. The per cent root colonization in sterilized and non sterilized soil was in the range of 80-89%.It may be concluded that, the combined application of *P. fluorescens* 7% WP as seed treatment @ 10 g/kg seed + soil treatment @ 2.5 Kg/ha or *P. fluorescens* 5% SC as seed treatment @ 10 ml/kg seed + soil treatment @ 2.5 lit/ha is suggested for effective management of bacterial wilt of chili.

***Keywords*— Chili, wilt, *Ralstonia solanecearum*, *Pseudomonas flurorescens*, biocontrol**

I. INTRODUCTION

Chilli (*Capsicum annum* L.) is one of the important vegetable and annual herbaceous spice crops in India. Chilli is grown in warm and humid climate of West Bengal. The sustainability of chilli cultivation has been threatened by a number of factors. Main biotic stress is wilt caused by the bacteria. Chilli is a universal spice of India. In India it is cultivated over an area of 9.15 lakh ha with an annual production of10.18 lakh tonnes of dry chilli (Anonymous, 2007). In recent years the focus has shifted to the control of diseases using bio-control agents, which are a safe and promising alternative to synthetic pesticides. There is some evidence that endophytes can contribute to the control of plant disease (Ramesh et al., 2009). In India, limited work has been done on the isolation of endophytic bacteria viz.,*Pseudomonas fluorescens* (Trevisan) Migula and *Bacillus subtilis* (Ehrenberg.) from stem and roots of chilli seedlings (Muthukumar, 2008); Bacillus sp., *P. fluorescens* and *Erwinia herbicola* (Dye.) from chickpea (Rangeshwaran et al., 2008). The internal tissues of plants provide a uniform and safe environment when compared to the rhizosphere and phylloplane where the introduced bacterial population must compete for nutrients. These advantages envisage the use of endophytic bacteria for more successful biological control of plant diseases (Sturz and Christie, 1995). Wilt of chilli caused by *Ralstonia solanacearum* is one of the important serious foliar diseases of chilli crop causing maximum crop losses during the Kharif and Rabi seasons in West Bengal. Hence, an eco-friendly approach for managing this disease through application of commercially formulated bio antagonist like *Pseudomonas fluorescens* has been employed, under *in vivo* condition for reducing degradation of soil health and environmental pollution. Keeping these views in mind present study was conducted with *Pseudomonas fluorescens* 7% WP and *Pseudomonas fluorescens* 5% SC against *Ralstonia solanacearum* giving special emphasis on bioefficacy, phytotoxicity, antagonistic activity and effect of bio antagonist on beneficial microbes.

II. MATERIALS AND METHODS

The experimental trials were conducted in University Instructional Farm during the successive cropping season of October, 2012 to February, 2013 and February, 2013 to June, 2013 with a susceptible crop cultivar Bullet sown in sandy loam soil having standard spacing (50cmx50cm) and plot size (2mx2m). All standard and recommended packages of agronomical practices such as tillage, spacing, manuring and irrigation for cultivation of the crop were followed during cultivation of crop. The ten treatments i.e., T_1=(*Pseudomonas fluorescens* 7% WP @ 10 g/kg seed)as seed treatment, T_2 =*(Pseudomonas fluorescens* 7% WP @ 2.5 kg/ha)as soil treatment, T_3= *Pseudomonas fluorescens* 7% WP @ 10g/kg seed + 2.5 kg/ha (as seed and soil treatment),T_4= *Pseudomonas fluorescens* 5% SC @ 10 ml/kg seed (as seed treatment), T_5= *Pseudomonas fluorescens* 5% SC @ 2.5 lit/ha (as soil treatment), T_6= *Pseudomonas fluorescens* 5% SC @ 10 ml/kg seed and 2.5 lit/ha as seed and soil treatment, T_7= Streptomycin sulphate

+ Tetracyclin hydrochloride (9:1) SP @ 100 ppm as foliar spray ,T_8= Untreated control (water only), T_9= *Pseudomonas fluorescens* 7% WP @ 20 g/kg seed + 5 kg/ha as seed and soil treatment for phytotoxicity evaluation, T_{10}= *Pseudomonas fluorescens* 5% SC @ 20 ml/kg seed + 5 lit/ha as seed and soil treatment for phytotoxicity evaluation were taken into consideration with four replications. Weather data on Temp. (Max. & Min.), Relative humidity (Morning and afternoon) and Rainfall for the experimental period were recorded from automatic weather station. Statistical data generated were transformed into arc sine values for statistical scrutiny, wherever necessary (Gomez and Gomez, 1984).

Disease scoring was done on 10 plants per plot through random selection. The present investigation was conducted during two cropping seasons of 2012 and 2013 in University Instructional Farm. The chilli genotype bullet was grown on raised bed with three replications. The observations for disease incidence were made for both bio-antagonist formulations applied as seed as well as soil treatment as per above mentioned dosages. The observations on disease incidence were recorded 20, 30 and 45 days after seedling transplantation uniformly in all the treatments. According to Chattopadhyay and Sen, 1996, the wilt intensity was recorded and classified into 0 – 3 scale (where 0= Healthy plants with no effect,1=Yellowing of lower leaves,2=Yellowing, marginal necrosis and defoliation,3=Drying and wilting/complete death of the plants) and per cent disease index (PDI) was calculated according to Wheeler (1969). The percentage value of data of disease severity was transformed to angular transformation and other data to square root transformation, wherever required, and analyzed statistically.

$$\text{PDI (\% disease index)} = \frac{\text{Sum of numerical ratings}}{\text{No. of plants observed x Max disease scored}} \text{x100}$$

For phytotoxicity evaluation the products were also applied at higher levels i.e. *Pseudomonas fluorescens* 7% WP @ 20 g/kg seed + 5 kg/ha soil application and *Pseudomonas fluorescens* 5% SC @ 20 ml/kg seed + 5 lit/ha soil application. Phytotoxicity observations were recorded after 7, 10, 12, 15 and 20 days of seed sowing and 1, 3, 7, 10 and 15 days after seedling transplanting. The phytotoxicity parameters observed were leaf injury on tips/surface, necrosis, vein clearing, wilting, epinasty and hyponasty. Leaf injury on tips/surface was rated based on 1 to 10 Scale (1=0-10%, 2=11-20%, 3=21-30%, 4=31-40%, 5=41-50%, 6=51-60%,7=61-70%,8=71-80%,9=81-90%,10=91-100%)as per Central Insecticide Board & Registration Committee guidelines(2011). For *In vitro* antagonistic activity study of *Ralstonia solanacearum* on the seed germination and seedling vigor index The antagonistic activity of *P. fluorescens* 7% WP and *P. fluorescens* 5% SC was tested *in vitro* against the pathogen by dual culture technique. The pathogenicity of *Rolstonia solanacearum* maintained in the laboratory on potato dextrose agar (PDA) medium was confirmed on chilli before testing the products against it. The test samples of the products were found to contain cfu 2.18×10^8 /g min. (*P. fluorescens* 7% WP) and 2.12×10^8 /g min. (*P. fluorescens* 5% SC). *P. fluorescens* was also isolated from the products using Pseudomonas Agar medium. The Petri dishes inoculated with the pathogen alone were taken as control. The Petri dishes were incubated under laboratory conditions (25 ± 2ºC). Four replications were maintained for each treatment. The inhibition zone in each treatment was recorded after 48 hrs of incubation. To observe the effect of products on the seed germination and seedling vigour of chilli another experiment was conducted using paper towel method (ISTA, 1985). The product *P. fluorescens* 7% WP was tested at four dosages viz. 5, 7.5, 10.0 and 12.5 g/kg seed and similarly *P. fluorescens* 5% SC was tested @ 5, 7.5, 10 and 12.5 ml/ kg seed. The required quantities of the products were mixed with 100 g chilli seeds thoroughly. One hundred seeds per replication were randomly picked from each treatment and such three replications per treatment were maintained. Ten seedlings per replication were randomly selected to record the data on root length, shoot length and dry weight of seedling after 14 days of treatment. Seedling vigour index was calculated using the following formula:

$$\text{Seedling vigor index} = (\text{Root length} + \text{Shoot length}) \times \text{percent seed germination}$$

Bioefficacy assay of antagonist *Pseudomonas fluorescens* 7% WP and *Pseudomonas fluorescens* 5% SC was done based on root colonization. The pathogen to be tested against was grown in sand maize medium. The sand-maize medium was prepared by adding sand 90g, maize 10g and water 10 ml in a saline or any glass bottle of 300ml capacity and then autoclaved twice. Then 5 discs of the test pathogen were transferred into the bottle and left for incubation for 15 days. Once the culture had grown well, the sand maize medium was mixed along with the pathogen and 1 g from this preparation was used as the inoculum after adjusting the cfu to 1 x 10/g by addition of sand.The plastic cups (5-6 cm diameter) filled with soil and FYM (3:1) were used. In each cup the filling was done upto ¾th level. The pathogen inoculum was mixed with sand and applied upto 2 cm depth in the plastic cups.

The bio efficacy of the bio agent was tested by both seed treatment and soil application. For seed treatment, the recommended dose of the formulation was used (10 g). For soil application, the bioagent was added at the rate of 1g of formulation (minimum cfu 2×10^6, the CIB recommended dose). For the root colonization assay, the rhizosphere region of the plants grown as above were collected and the soil adhering to the root surface was removed by gently tapping the roots. The root bits were cut into 1 cm bits and randomly 25 bits were selected for each treatment. These were plated on TSM and the percentage of root bits colonized was recorded. This was performed in the sterile soil and not sterile soil. One control treatment without the biocontrol agent being tested was kept for both the sterile and non-sterile soil to rule out the possibility of interference of native microflora in the bioefficacy assay.

For investigating the effect of *Pseudomonas fluorescens* 7% WP and *Pseudomonas fluorescens* 5% SC on soil beneficial micro-organisms in chili crop**,** The population of soil micro-organisms was recorded before seed sowing and

7 and 14 days thereafter. Similarly in transplanted crop also the observation was recorded before transplanting the seedlings and 7 and 14 days thereafter.

III. Results and Discussion

The result in Table 1 reveals that during the trial of bio pesticide, *P. fluorescens* 7% WP and *P. fluorescens* 5% SC applied as seed treatment significantly increased the germination percentage (86.75 to 90.50%) as compared to non treatment of seeds (74.25 to 78.25%). All the *P. fluorescens* treatments at each level of application and Streptomycin sulphate + Tetracyclin hydrochloride (9:1) SP @ 100 ppm were significantly effective to reduce the disease development under field condition as compared to untreated control at 30 and 45 days after transplanting the crop. It was further observed that combined approach of seed treatment and soil treatment were most effective (0.83 to 10.82% PDI) than either seed treatment or soil treatment alone (3.33 to 24.98% PDI). The control treatment showed highest disease incidence (9.99 to 44.96% PDI).Present observation also makes conformity with Kloepper et al (2004) also described that PGPR strains have ability to suppress the disease by inducing systemic disease resistance in plants against broad spectrum phyto pathogens. Elabadry et al (2006) also observed significant reduction in percent disease intensity and virus concentration in plants treated with *Pseudomonas fluorescens* and *Rhizobium leguminosarum* as compared to the control.

Table 1. Effect of seed and soil treatment with bio pesticide *Pseudomonas fluorescens* 7% WP and *Pseudomonas fluorescens* 5% SC against bacterial wilt disease of chilli under field condition.

Treatment with doses	Seed Germination (%)	Disease incidence (%) 20 DAT	30 DAT	40 DAT
P. fluorescens 7% WP(10 g/kg of seed) (T_1)	88.50	3.33 (10.54)	10.82 (19.52)	20.81 (27.42)
P. fluorescens 7% WP(2.5 kg/ha) (T_2)	78.25	6.66 (15.31)	13.32 (21.36)	24.98 (30.16)
P. fluorescens 7% WP(10 g/kg seed + 2.5 kg/ha) (T_3)	90.50	1.67 (7.67)	5.00 (13.40)	10.82 (19.52)
P. fluorescens 5% SC(10 ml/kg seed) (T_4)	87.50	4.16 (11.60)	11.66 (20.36)	24.14 (29.64)
P. fluorescens 5% SC(2.5 lit/ha) (T_5)	74.25	5.83 (14.25)	9.16 (17.54)	21.65 (27.80)
P. fluorescens 5% SC(10 ml/kg seed + 2.5 lit/ha) (T_6)	86.75	0.83 (5.86)	5.83 (14.46)	9.99 (18.46)
Streptomycin sulphate + Tetracyclin hydrochloride (9:1) SP @100 ppm (T_7)	75.50	4.16 (11.60)	9.99 (18.78)	15.82 (23.18)
Untreated control (T_8)	76.00	9.99 (18.78)	21.65 (27.80)	44.96 (42.36)
$SE_m\pm$	1.69	(2.19)	(1.94)	(2.42)
CD (P=0.05)	4.98	(6.44)	(5.72)	(7.12)

Figures in parentheses are angular transformed values
DAT – Days after transplanting

The vegetative growth as well as reproductive yield of chilli were significantly influenced by combination of seed and soil treatments with *P. fluorescens* 7% WP @ 10 g/kg seed + 2.5 kg/ha as well as with *P. fluorescens* 5% SC applied @ 10 ml/kg seed + 2.5 lit/ha (Table-2).

Table 2. The effect of bio pesticide (*Pseudomonas fluorescens* 7% WP formulation and *Pseudomonas fluorescens* 5% SC formulation) against vegetative and reproductive yield of chilli under field condition

Treatment with Doses	Fresh root wt (g)	Dry root wt (g)	Fresh shoot wt (g)	Dry Shoot wt (g)	Plant height (cm)	Yield (kg/ha)
P. fluorescens 7% WP(10 g/kg of seed) (T_1)	6.23	2.05	57.35	23.28	42.23	235.03
P. fluorescens 7% WP(2.5 kg/ha) (T_2)	5.70	1.73	53.03	21.65	38.05	213.58
P. fluorescens 7% WP(10 g/kg seed + 2.5 kg/ha) (T_3)	7.43	2.80	60.23	26.33	47.53	265.60
P. fluorescens 5% SC(10 ml/kg seed) (T_4)	5.40	1.53	50.20	20.78	39.33	230.28
P. fluorescens 5% SC(2.5 lit/ha) (T_5)	5.03	1.20	48.35	17.80	36.45	218.38
P. fluorescens 5% SC(10 ml/kg seed + 2.5 lit/ha) (T_6)	6.63	2.53	55.45	24.25	44.83	257.18
Streptomycin sulphate + Tetracyclin hydrochloride (9:1) SP @100 ppm (T_7)	4.55	1.58	54.05	22.75	35.80	244.15
Untreated control (T_8)	4.10	1.13	34.98	15.53	30.23	182.53
$SE_m\pm$	0.14	0.13	1.26	0.98	1.35	9.00
CD (P=0.05)	-0.42	0.38	-3.69	2.88	3.97	26.46

The seed treatment followed by soil treatment exhibited best result in terms of fresh root weight (6.63 g), dry root weight (2.53 g), fresh shoot weight (55.45 g), dry shoot weight (24.25g), plant height (44.83cm) and yield (257.18 kg/ha) than control and other treatments. Hence, *P. fluorescens* 7% WP @ 10 g/kg seed + 2.5 kg/ha soil treatment and *P. fluorescens* 5% SC @ 10 ml/kg seed and 2.5 lit/ha soil treatment were effective for the control of bacterial wilt disease and increase in yield of chilli. The significant yield increase was noticed due to application of PGPR as seed and soil treatment. The present result makes conformity with Minorsky 2008, where he showed that use of PGPRs enhanced the plant growth, seed emergence and overall yield of crops in different agro-ecosystems. Inoculation of PGPR species could increase the growth attributes like leaf area, chlorophyll content and consequently, the total biomass of the available nutrients by PGPR.

The phytotoxic symptoms like leaf injury on tips/surface, necrosis, vein clearing, wilting, epinasty and hyponasty were not observed due to the application of different dosages of *P. fluorescens* 7% WP and *P. fluorescens* 5% SC in nursery as well as in transplanted crop.

Table 3. The effect of bio pesticide (*P. fluorescens* 7% WP formulation and *P. fluorescens* 5% SC formulation) on soil beneficial organisms

Treatment with doses	Nursery Chilli					
	Mean *Rhizobium* sp. (1 x 10^4 cfu/g soil)			Mean *Azotobacter* sp. (1 x 10^5 cfu/g soil)		
	PRE	7 DAS	14 DAS	PRE	7 DAS	14 DAS
P. fluorescens 7% WP(10 g/kg of seed) (T_1)	11.42	13.42	16.39	8.22	10.27	18.21
P. fluorescens 7% WP(2.5 kg/ha) (T_2)	-	-	-	-	-	-
P. fluorescens 7% WP(10 g/kg seed + 2.5 kg/ha) (T_3)	-	-	-	-	-	-
P. fluorescens 5% SC(10 ml/kg seed) (T_4)	9.52	11.59	18.86	7.63	9.84	17.75
P. fluorescens 5% SC(2.5 lit/ha) (T_5)	-	-	-	-	-	-
P. fluorescens 5% SC(10 ml/kg seed + 2.5 lit/ha) (T_6)	-	-	-	-	-	-
Streptomycin sulphate + Tetracyclin hydrochloride (9:1) SP @100 ppm (T_7)	12.83	10.91	17.37	7.31	11.49	15.72
Untreated control (T_8)	10.82	12.21	19.41	8.56	10.27	19.79

-Not evaluated PRE- Pre treatment

Table 4. The effect of bio pesticide (*P. fluorescens* 7% WP formulation and *P. fluorescens* 5% SC formulation) on soil beneficial organisms

Treatment with doses	Transplanted chilli					
	Mean *Rhizobium* sp. (1 x 10^5 cfu/g soil)			Mean *Trichoderma* sp. (1 x 10^6 cfu/g soil)		
	PRE	7 DAT	14 DAT	PRE	7 DAT	14 DAT
P. fluorescens 7% WP(10 g/kg of seed) (T_1)	3.28	5.32	8.33	13.44	12.67	23.42
P. fluorescens 7% WP(2.5 kg/ha) (T_2)	5.41	6.16	9.43	10.81	13.35	20.30
P. fluorescens 7% WP(10 g/kg seed + 2.5 kg/ha) (T_3)	7.12	5.93	8.54	11.48	12.79	18.64
P. fluorescens 5% SC(10 ml/kg seed) (T_4)	6.82	5.70	9.56	10.94	15.26	17.45
P. fluorescens 5% SC(2.5 lit/ha) (T_5)	5.47	7.35	8.06	9.58	13.74	19.73
P. fluorescens 5% SC(10 ml/kg seed + 2.5 lit/ha) (T_6)	8.53	8.26	10.24	12.46	12.69	21.62
Streptomycin sulphate + Tetracyclin hydrochloride (9:1) SP @100 ppm (T_7)	6.75	7.97	9.71	14.47	16.68	23.17
Untreated control (T_8)	4.48	8.36	10.86	13.73	19.34	22.47

PRE- Pre treatment

The result in Table 3 & Table 4 shows that the populations of *Rhizobium* sp., *Azotobacter* sp. and *Trichoderma* sp. were more or less similar in various treatments. It showed that there was no adverse effect of *P. fluorescens* 7% WP and *P. fluorescens* 5% SC on soil beneficial micro-organisms.

The *P. fluorescens* 7% WP and *P. fluorescens* 5% SC inhibited the growth of the pathogen effectively ranging from 15 mm to 22 mm with an average of 17.25 mm and 17.75 mm, respectively (Table-5). Therefore, it confirms the pathogenicity of the products against the pathogen. Significant antagonistic activity was shown by the test product. The present observation also makes conformity with previous worker. Jing et al (2007) reported that, Rhizobacteria can inhibit the growth of several phytopathogens in several ways like competing for nutrient and space, limiting availability of Fe supply through producing siderophore, producing lytic enzymes and antibiosis.

Table 5. Effectiveness of *P. fluorescens* 7% WP and *P. fluorescens* 5% SC on the growth of *Rolstonia solanacearum* under in vitro condition

Treatment	Inhibition zone (mm)				
	R_1	R_2	R_3	R_4	Average
P. fluorescens 7% WP	17	16	21	15	17.25
P. fluorescens 5% SC	22	18	16	15	17.75
Control	0.00	0.00	0.00	0.00	0.00

Table 6. Effectiveness of *P. fluorescens* 7% WP and *P. fluorescens* 5% SC on the seed germination, root and shoot length, dry weight of seedling, and seedling vigour index of chilli

Treatment	Dose (g or ml/ kg seed)	Mean seed germination (%)	Mean root length (cm)	Mean shoot length (cm)	Mean dry wt. of seedling (mg)	Seedling vigor index
P. fluorescens 7% WP	5.0	86.33 (68.32)*	4.80	6.40	12.863	967.23 (31.10)**
P. fluorescens 7% WP	7.5	87.67 (69.50)	5.23	6.47	13.920	1025.33 (32.02)
P. fluorescens 7% WP	10.0	91.00 (72.60)	5.93	7.23	16.060	1198.40 (34.61)
P. fluorescens 7% WP	12.5	91.67 (73.26)	5.87	7.30	16.813	1207.37 (34.74)
P. fluorescens 5% SC	5.0	86.00 (68.06)	4.83	6.33	12.897	960.33 (30.99)
P. fluorescens 5% SC	7.5	88.33 (70.05)	5.17	6.53	13.237	1033.83 (32.14)
P. fluorescens 5% SC	10.0	92.33 (74.05)	5.83	7.33	16.350	1215.77 (34.87)
P. fluorescens 5% SC	12.5	91.00 (72.61)	5.90	7.37	16.873	1207.53 (34.74)
Control	–	81.67 (64.68)	4.53	5.47	10.017	816.37 (28.57)
S.Em ±		(1.08)	0.12	0.16	0.22	(0.40)
C.D. (P = 0.05)		(3.24)	0.35	0.48	0.66	(1.19)

*Figures are angular transformed values

** Figures are square root transformed values

The application of *P.fluorescens* as seed treatment significantly increased the root length, shoot length, dry weight of seedling and seedling vigour index in all the treatments as compared to control (Table-6).

P.fluorescens 7%WP @ 12.5g/Kg and *P.fluorescens* 5%SC @12.5 ml/Kg showed best results here in all respect. Sarvanakumar and Samiyappan (2007) also found that total root length, surface area and volume in tomato and cucumber roots increased after inoculation with *P.fluorescens* 92rk and P190r.

The percent root colonization in sterilized and nonsterilized soil was found in the range of 84-92% (Table - 7) which is more than the requirement of 80% by CIB &RC (2011)

Table 7: Per cent root colonization in chilli by *P. fluorescens* 7% WP and *P. fluorescens* 5% SC

Treatment	Per cent root colonization							
	In sterilized soil				In non sterilized soil			
	R1	R2	R3	Mean	R1	R2	R3	Mean
Pseudomonas fluorescens 7% WP	80	88	84	84.00	88	84	92	88.00
Pseudomonas fluorescens 5% SC	84	84	88	85.33	88	92	88	89.33
Control	0	0	0	0	4	0	8	3.00

IV. CONCLUSIONS

Hence, it may be concluded that, the application of biocontrol agents as seed treatments could prove to be a beneficial component of integrated plant disease management. These *P. fluorescens* isolates, apart from their action against bacterial wilt pathogen, are good growth promoters. It is evident that rhizobacteria could possibly serve as ecofriendly and sustainable alternatives to the hazardous chemicals used for growth promotion and management of plant diseases.

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