

***In vitro* Studies on *Colletotrichum falcatum* the Causal of Red Rot Disease of Sugarcane**

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Abstract: Red rot caused by *Colletotrichum falcatum* is one of the most destructive disease of sugarcane. The effect of Relative Humidity (RH), pH, temperature, water, media, plant extracts, smokes and fungicides were studied on the Conidial Germination (CG) and Mycelial Growth (MG) of *C. falcatum*. The highest CG and MG was observed at 6-8 pH, 90-95 RH and 25-30°C. Pond and distilled water were favorable (46%) and unfavorable (17%) for CG, respectively. Concentration of 2-3% glucose and sucrose were the best for CG and MG and sucrose was better than glucose. Among 7 solid media, the highest MG was 73 mm found on oatmeal agar medium and the lowest was 12 mm on Czapek's medium. Leaf extracts of *Curcuma domestica* and *Datura metel* and bark extracts of *Swietenia mahagoni* were suitable for both Percent Inhibition of Conidial Germination (PICG) and Percent Inhibition of Mycelial Growth (PIMG) of the pathogen. Smoke of dhup (incense) and tobacco leaf were showed good PICG. Dithane M-45, bavistin and redomil were found to be the best for PICG of tested fungus. The present study suggests that the growth and proliferation of red rot pathogen is exhibitedly affected by different environmental and nutritional conditions and the application of plant extracts and smokes instead of fungicides may play an active inhibitory role on the fungus.

Key words: *Colletotrichum falcatum* • fungicides • inhibition • plant extracts • red rot • smoke

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a major industrial cash crop and main source of sugar in Bangladesh. Some chemicals and industrial products such as alcohol, paper and paperboard are manufactured from the by products of sugarcane. The cultivation of sugarcane is widespread in tropical areas worldwide in a total of 19.4 million hectares as a single crop [1]. Sugarcane can also be intercropped with smaller plants such as potato, maize, sunflower and beans in Asia, Africa and Brazil [2]. About hundred diseases of sugarcane have been reported from different parts of the world which are obstacle to get well product of this crop [3]. Red rot of sugarcane is one of the most important fungal disease in Bangladesh [4]. This disease was first reported from Java, Indonesia [5] and generally known to cause by the fungus, *Colletotrichum falcatum* Went. The perfect stage of the fungus was identified as *Phylospora tucumanensis* Speg. [6] and finally known as *Glomerella tucumanensis* Speg. [7]. Red rot disease is mainly responsible for the deterioration of many promising commercial varieties and continues to be a problem in mainland U.S.A., Taiwan, Bangladesh, India, Australia

and other countries. [8]. Alone this disease is responsible for about 10-15 per cent yield reduction. It causes losses in several ways, such as germination failure, poor sprouting of stubble, death of tillers and cane stalks.

Sugarcane crop remains in the field for 10-12 months passing through various environmental conditions. Growth and proliferation of red rot pathogen is influenced by different environmental and nutritional conditions and the application of plant extracts, smokes and fungicides may play an active inhibitory role on the fungal movement. In the present study, two attempts have been taken. First, to find out the favorable environmental and nutritional conditions for this fungus and second, to select the best antifungal plant extract, smoke and fungicide which have good inhibitory effect against *C. falcatum* pathogen of red rot disease of sugarcane.

MATERIALS AND METHODS

Pathogen: *Colletotrichum falcatum* was isolated from stem tissue of infected sugarcane and cultured on oatmeal agar medium (Fig. 1). Ten days old culture of pathogen was used for each experiment.

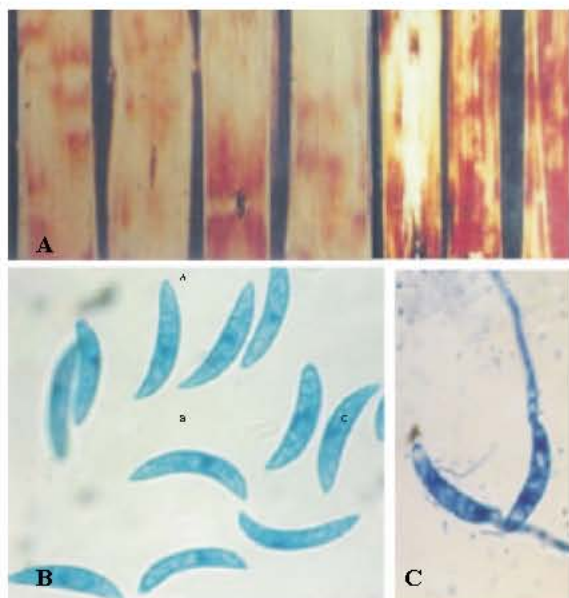


Fig. 1: A. Longitudinal section of infected stem of sugarcane, B. Conidia and C. Germinated conidia of *C. falcatum*

Environmental and nutritional factors

Conidial Germination (CG): Relative humidity (70, 75, 80, 85, 90, 95 and 100), pH (4, 5, 6, 7, 8, 9 and 10), temperatures (5, 10, 15, 20, 25, 30 and 35°C), water collected from 7 sources (distilled, pond, rain, river, sea, tap and tube well water), glucose and sucrose solutions (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5% concentrations) were used to get suitable condition for CG of *C. falcatum*. Conidia from the 10 days old culture of oatmeal agar plate were taken and conidial suspensions (10^3 /ml) were made with sterilized distilled water (for RH, pH and temperature)/ 7 sources of water/ different concentrations of glucose and sucrose solution separately. The conidial suspension was taken in sterilized watch glass, a drop of conidial suspension was taken on separate grove slide and kept at 25°C in a moisture chamber for 24 hours. After incubation period a drop of lactophenol cotton blue was placed over conidial suspension on the slide and examined under ($\times 400$) power microscope for recording the percentage of CG.

Mycelial Growth (MG): Relative humidity, pH, temperature, glucose, sucrose (same range as CG) and 7 solid media {oatmeal, richard's, PDA (potato dextrose agar), host (extract of sugarcane), sabouraud's, PCM (paper chromatography medium) and czapec's} were used to get favorable MG of *C. falcatum*. Effect of RH, pH, temperature, glucose and sucrose on MG of *C. falcatum*

was done using oatmeal agar medium. Different media were autoclaved at 121°C/15lbs/inch² pressures, these were poured in sterilized Petri dishes. Agar discs (5 mm) were taken from 10 days old culture of *C. falcatum* and placed separately in respect to each treatment in the center of each petri dish and incubated respective condition. After 10 days of incubation radial growth of mycelium was measured by following Brown methods [11]. Three replicates were used for each particular treatment.

Extraction of plants and application: Seed of *Azadirachta indica*, leaf of *Curcuma domestica*, *Datura metel*, *Ocimum sanctum* and bark of *Swietenia mahagoni* was extracted following the method described by Mahadevan and Sridhar [9]. Plant materials were cut into pieces and immediately plunged in sterilized distilled water (1g: 10ml) in a beaker and allowed to boil for 5~10 minutes. The tissues were crushed thoroughly in a mortar with a pestle and then passed through two layers of cheesecloth. For re-extraction, the ground tissues were boiled again with sterilized distilled water (1g: 10ml) for 3~5 minutes. The extracts were then cooled, passed through cheesecloth and filtered through Whatman's no. 1 filter paper. Extracts were evaporated on a steam bath to dryness and made 5% (0.05 g/ml) concentration with sterilized distilled water for experiment. Conidia of *C. falcatum* were taken from 10 days old culture of oatmeal agar medium and treated for 20 minutes with each plant extract separately. A drop of plant extract treated conidial suspension was taken on slide, removed the plant extract using blotting paper, added a drop of sterilized water and incubated in moisture chamber at 25°C for 24 hours. After incubation period a drop of lactophenol cotton blue was placed over conidial suspension on the slide and examined under ($\times 400$) power microscope for recording the percentage of CG.

Application of smokes: The evaluation of fungitoxicity of smoke in the laboratory was made by Parmeter's technique [10] with a modification. Dhup (incense), rice straw, sawdust, tobacco leaf and wheat straw were burnt in the metal pot with a cover fitted with rubber tube. The resulting smoke was cooled to ambient temperature by passing through the rubber tube. Uncovered Petri plates containing the fungal culture grown on oatmeal medium were placed for 20 minutes into the smoke chamber (a wooden box of 0.5 m \times 0.5 m \times 0.5 m, where smokes are passed through by rubber tube) and exposed to cool dense dhup, rice straw, sawdust, tobacco leaf and wheat straw smoke. Aqueous conidial suspension

(10^3 conidia/ml) of the pathogen was placed on grove slides previously exposed to dhup, rice straw, sawdust, tobacco leaf and wheat straw smoke then incubated in a moist chamber for 24 hours. For control, CG was counted in aqueous suspension (without treated) made with sterilized distilled water. After incubation period slides were examined as similar manner.

Application of fungicides: Five different fungicides such as bavistin (Methyl-2 benzimidazole carbamate), cupravit (copper oxychloride), dithane M-45 (Complex of zinc and maneb containing 20% manganese and 2.5% zinc), redomil {(Methyl-2 benzimidazole carbamate-N-2-methobis acetyl, alaninite; zinc manganese ethylene bis (dithiocarbamate)} and thiovit (poly sulphide) were used at 1000 ppm concentrations. Conidia of *C. falcatum* were taken from 10 days old culture of oatmeal agar medium and conidial suspensions (10^3 conidia/ml) were made with each fungicidal suspension (1000 ppm). These suspensions (5 ml) were taken in sterilized watch glass and kept at 25°C for 20 minutes. A drop of fungicidal treated conidial suspension was taken on slide, removed the fungicidal solution using blotting paper, added a drop of sterilized water and kept in moisture chamber at 25°C for 24 hours of incubation. Then a drop of lactophenol cotton blue was placed over conidial suspension on the slide. For control, CG was counted in sterilized distilled water. The slides were examined similarly.

Inhibition: Five different plants extracts {for Percent Inhibition of Conidial Germination (PICG), Percent Inhibition of Mycelial Growth (PIMG) and Percent Inhibition of Mycelial Weight (PIMW)} smokes and fungicides (for PICG) were observed for testing antifungal activity. For observing PICG, CG in plant extracts, fungicides and smokes (R_2) were recorded described as earlier. For control, CG was counted in non-treated respective condition (R_1). Three replicates were used for each case. The two readings were transformed into PICG using the formula Skidmore and Dickinson [12], where $PICG = (R_1 - R_2) / R_1 \times 100$.

Determining the PIMG and PIMW, plant extracts were diluted at 5% (0.05g/ml) concentration with PDA and PDB (potato dextrose broth) medium separately. After autoclaving at 121°C for 15 minutes, PDA was poured in sterilized Petri dishes. Agar discs (5 mm) were taken from 10 days old cultures of *C. falcatum* and placed in the center of the Petri plates separately. As control, fresh PDA was inoculated with the test fungus. All Petri plates with tree replicates were incubated at 25°C for 10 days.

The PIMG was assessed by measuring the MG grown in plant extracts medium (R_2) and the MG grown in fresh PDA as control (R_1). The two readings were transformed into PIMG using the same formula [12].

In case of PIMW, 100 ml of liquid medium (PDB) in 250 ml of conical flask was inoculated with *C. falcatum* and incubated on rotary shaker 140-150 rpm at 25°C separately. As control, fresh PDB was inoculated with the test pathogen and incubated similarly. After 10 days of incubation, the entire fungal mycelium was harvested by filtering through previously dried and weighted Whatman filter paper No 1. It was then dried to constant weight at 65°C. Before weighting, the filter paper were allowed to cool and subsequently weighted in a balance. The weight of the mycelium was calculated by deducting the weight of filter paper from the final weight. All pairings were carried out in 3 replicates. The PIMW was assessed by measuring the Mycelial Weight (MW) grown in plant extracts (R_2) and the MW grown in fresh PDB as control (R_1). The two readings were transformed in to PIMW using the same formula [12].

RESULTS AND DISCUSSION

Environmental and nutritional factors affecting CG and MG of *C. falcatum* were observed. The highest CG was 81% at pH 7, 73% at RH 95 and 70% at 25°C. Water collected from 7 sources was experimented as a source of nutrient. Among them, the highest 46% CG was found in pond water and the lowest was 17% in distilled water. River, rain and sea water were also good for CG. Different concentrations of glucose and sucrose were tested on CG and in both cases 2.5% solution was the best. In this concentration, the highest CG was 46 and 60% in glucose and sucrose solution, respectively (Table 1). Chauhan and Singh [13] found that zoospores of *Phytophthora drechsleri* f. sp. *cajani* germinated in a wide range of pH (4-11). The maximum percentage of germination was observed at pH 7.5. Effect of RH on CG of chilli fruit rot pathogen *Alternaria tenuis* has been reported and observed that CG was initiated in 2-6 hours at 90 and 75% relative humidity [14]. The germination of ascospore of *Mycosphaerella musicola* takes place at RH 88% [15]. Lim and Tang [16] studied the growth and sporulation of *Colletorichum gloeosporioides* on oatmeal agar are most abundant at 80-90% of RH. Singh and Chauhan [17] found that maximum germination of zoospore of *Phytophthora drechsleri* was at 30°C and minimum was at 10 and 45°C. Rewal and Grewal [18] reported that minimum, optimum and maximum temperature for the

Table 1: Effect of different environmental and nutritional factors on the conidial germination of *C. falcatum*

pH	% of conidial germination after 24 h									
	CG	RH (%)	CG	°C	CG	Water	CG	Glucose/Sucrose (%)	CGG	CGS
4	41	70	13	05	00	Distilled	17	0.5	21	25
5	55	75	19	10	29	Pond	46	1.0	26	28
6	72	80	27	15	37	Rain	25	1.5	37	42
7	81	85	39	20	52	River	33	2.0	43	56
8	69	90	72	25	70	Sea	29	2.5	46	60
9	45	95	73	30	68	Tap	22	3.0	44	51
10	29	100	56	35	51	Tube well	20	3.5	37	43

RH: Relative humidity, °C: Temperature, CG: Conidial Germination, CGG: Conidial Germination in Glucose, CGS: Conidial Germination in Sucrose. All treatments were observed (except temperature effect) at 25°C

Table 2: Effect of different environmental and nutritional factors on the mycelial growth of *C. falcatum*

pH	Mycelial growth (mm) after 10 days									
	MG	RH (%)	MG	°C	MG	Media	MG	Glucose/Sucrose (%)	MGG	MGS
4	28	70	33	05	00	Czapek's	12	0.5	31	42
5	39	75	48	10	27	Host	38	1.0	45	66
6	63	80	52	15	36	Oatmeal	73	1.5	63	79
7	64	85	55	20	48	PCM	18	2.0	84	85
8	52	90	59	25	69	PDA	54	2.5	89	90
9	49	95	57	30	77	Richard's	56	3.0	90	90
10	42	100	54	35	56	Sabouraud's	23	3.5	87	88

RH: Relative Humidity, °C: Temperature, MG: Mycelial Growth, MGG: Mycelial Growth in Glucose, MGS: Mycelial Growth in Sucrose. All treatments were observed (except temperature effect) at 25°C

germination of conidia of all the three strains of *Botrytis cinerea* were 9°, 20° and 30°C. The spores of *C. gloeosporioides* germinate maximum in tap water [19]. The conidia of *Mycosphaerella fijiensis* var. *difformis* germinate maximum in free water [20]. Appaji and Thakur [21] reported *in vitro* experimental result of pearl millet downey mildew by *Sclerospora graminicola* that the effect of dextrose (47.1%) and maltose (46.6%) were similar, so was sucrose (43.1%) and mannose (41.5%). Glucose alone was significantly different from rest of the sugars and it did not differ significantly with control. It induced higher percent of germination (52.9).

The highest MG was 64, 59 and 77 mm at pH 7, RH 90 and 30°C, respectively. Among 7 media, the highest MG was 73 mm found on oatmeal agar medium and the lowest was 12 mm on Czapek's medium. Host, PDA and Richard's media were also good for MG. In different concentrations of glucose and sucrose solution, the highest MG was 90 mm both in 2.5 and 3.0% of glucose and sucrose solution, respectively (Table 2). Satya and Grewal [22] found that *F. caeruleum* grew at different pH levels ranging from 2-8. No growth was obtained at pH 2. Optimum growth of the fungus was recorded at pH 6.

Mathur and Sorbhoy [23] recorded the maximum growth and excellent sporulation of *Sclerotium rolfsii* and *Alternaria alternata* at pH 5.5. Kore and Kharwade [24] studied the effect of RH on the growth and sporulation of *F. oxysporum* causing fruit rot of round gourd on PDA. They observed that RH of 70-90% shows good growth of the fungus, whereas growth is retarded at 100% RH. They also observed that 30 and 50% RH gives poor growth of the fungus. Bedi and Singh [25] observed that growth of *A. alternata* took place between 5-40°C with the optimum at 30°C, below 10° and above 35°C the growth was very slow. The growth was completely suppressed at temperature below 5°C and above 40°C. The pathogen sporulated at a temperature range of 10-30°C with maximum sporulation at 25°C. Quaroshi and Meah observed that *Botryodiplodia theobromae* grows faster on Richard's agar medium and poorest on Czapek's agar medium [26].

Plant extracts, smokes and fungicides were tested on PICG, PIMG and PIMW of *C. falcatum*. The highest PIMG and PIMW was 82.0 and 78.7 in *S. mahagoni* bark respectively but the PICG was the highest 98.3 in *D. metel* leaf extract medium presented in

Table 3: Inhibitory effect of different plant extracts, smokes and fungicides applied on *C. falcatum*

Plant extracts	PICG	PIMG	PIMW	Fungicides	PICG	Smokes	PICG
<i>A. indica</i> seed	85.0	37.3	31.4	Bavistin	97.6	Dhup	94.4
<i>C. domestica</i> leaf	88.3	71.6	66.2	Cupravit	85.2	Rice straw	62.5
<i>D. metel</i> leaf	98.3	77.6	72.9	Dithane M-45	98.9	Sawdust	79.7
<i>O. sanctum</i> leaf	78.0	29.8	36.1	Redomil	94.0	Tobacco leaf	91.2
<i>S. mahagoni</i> bark	98.2	82.0	78.7	Thiovit	79.5	Wheat straw	49.2

Percent Inhibition of Conidial Germination (PICG) was counted after (conidia were treated with plant extract, fungicide and smoke for 20 minutes separately) 24 h of incubation in sterilized distilled water at 25°C, Percent Inhibition of Mycelial Growth (PIMG) and Percent Inhibition of Mycelial Weight (PIMW) was measured after 10 days of incubation at 25°C. Plant extract and fungicide was used at 0.05g/ml and 1000 ppm, respectively

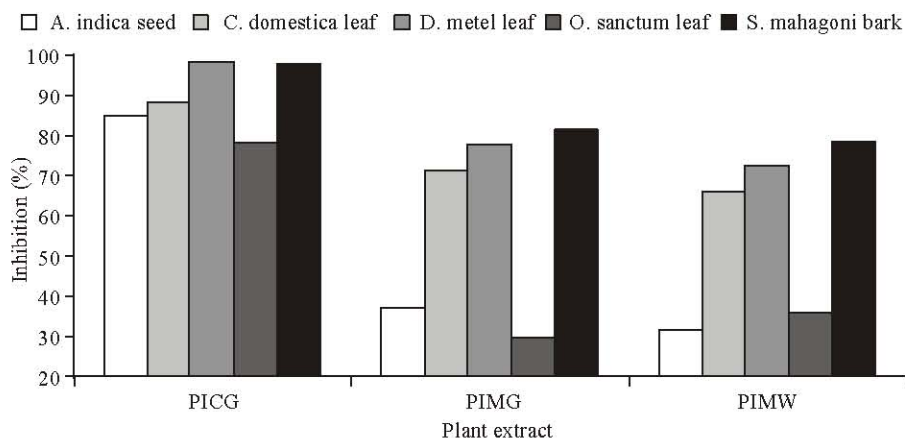


Fig. 2: Inhibitory effect of plant extracts. Percent Inhibition of Conidial Germination (PICG) was counted after (conidia were treated with plant extract at 0.05g/ml concentration for 20 min) 24 h of incubation in sterilized distilled water, Percent Inhibition of Mycelial Growth (PIMG) and Percent Inhibition of Mycelial Weight (PIMW) was measured after 10 days of incubation at 25°C

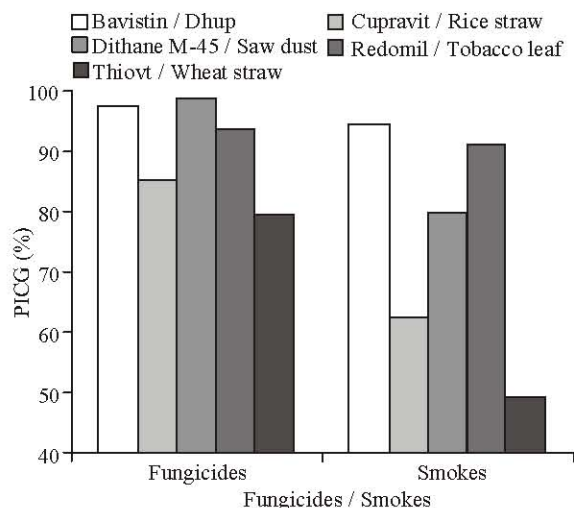


Fig. 3: Inhibitory effect of fungicides and smokes. Percent Inhibition of Conidial Germination (PICG) was counted after (conidia were treated with fungicide 1000 ppm and smoke for 20 min separately) 24 h of incubation in sterilized distilled water at 25°C

Table 3 and Fig. 2. Among 13 plant extracts, *Curcuma longa* (leaf and rhizome), *Tagetes erecta* (leaf) and *Zingiber officinales* (rhizome) were completely inhibited the CG of *C. gloeosporioides* after 15 minutes of incubation [27]. Alam *et al.* tested five plants extracts (leaf extracts of *Ocimum sanctum*, *Lantana camara*, *Calotropis procera*, *Azadirachta indica* and *Vinca rosea*) against CG of *Piper betle* root rot pathogen (*Fusarium oxysporum*) and got more or less inhibitory effect [28]. The effect of ethanolic extracts of *Lawsonia inermis*, *Azadirachta indica*, *Vinca rosea*, *Tagetes patula*, *Ocimum sanctum*, *Colocasia antiquarum*, *Adhatoda vasica*, *Moringa olefera*, *Datura metel* and *Curcuma longa* leaf on CG, MG and sporulation of *Aspergillus flavus*, *A. nigr*a and *A. fumigatus*. They reported all the tested plant extracts have a more or less inhibitory effect on CG, MG and sporulation on the selected fungi [29].

Except thiovit, all of tested fungicides were showed good inhibitory effect on PICG. The PICG was recorded 97.6, 85.2, 98.9, 94.0 and 79.5 in bavistin, cupravit, dithane

M-45, redomil and thiovit, respectively (Table 3 and Fig. 3). Dithane M-45 and redomil was highly effective to inhibit the CG of *C. gloeosporioides* pathogen of mango anthracnose disease when conidia were immersed for 10–20 minutes at 500–1000 ppm [27]. The effect of fungicides studied on the inhibition of *Bipolaris sorokiniana* and found Bavistin, Dithane M-45 tilt to be the most effective fungicides. They stated that concentrations of 500 to 2500 ppm and 1/10 to 1/1000 ml were the most effective after 5 to 30 minutes immersion [30].

In case of smokes, dhup and tobacco leaf was the best and PICG was 94.4 and 91.2, respectively. Smoke of sawdust was better than the smoke of rice and wheat straw (Table 3 and Fig. 3). Smoke of dhup has good inhibitory effect on CG of *F. oxysporum* [28]. Alam *et al.* stated that smoke of rice straw, wheat straw, tobacco leaf and dhup had a great effect against *B. sorokiniana*, *F. oxysporum* f. sp. *vasinfectum*, *R. artocarpi* and *B. theobromae* [31]. The growth of *A. tenuis* pathogen of chili fruit rot was totally inhibited when inoculated on the medium exposed to rice straw smoke and dhup smoke for 5–15 minutes [32].

CONCLUSION

The present research focused two results, first, is the optimal environmental and nutritional conditions for the pathogen, based on this result farmers may be able to escape the disease. And second, is the screening of plant extract, smoke and fungicide which have good inhibitory effect on the *C. falcatum*. Needless to say that use of chemical fungicides is a conventional method to control the fungal disease but it is the cause of tremendous environmental pollution and health hazard of animal kingdom. This study suggested that the tested plant extracts and smokes have antifungal effect against the red rot pathogen. Application of these plant extracts and smokes is environmentally sustainable and will reduce the fungal disease resulting production of sugarcane will be increased in Bangladesh.

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