

# Efficacy of Certain Essential Oils against Fusarium oxysporum and Colletotrichum capsici Caused Wilt and Anthracnose Diseases to Chilli

\*1Kavita Shelke and 2Dr. NS Suryawanshi

### **Abstract**

The antifungal activity of essential oils were studied *in vitro* against *Fusarium oxysporum f. sp. capsici* Schlecht. Emend. Snyder & Hansen and *Colletotrichum capsici* (Syd.) E. J. Butler & Bisby, both pathogens isolated from rotted chilli (*Capsicum annuum* L.). *F. oxysporum* and *C. capsici* were sever pathogens on chilli which were caused Fusarium wilt and anthracnose disease respectively. The use of essential oils for the management of Fusarium wilt and anthracnose consider to be a non-hazardous to environment and human beings. Twenty different essential oils were tested for their antifungal activity against *F. oxysporum* and *C. capsici in vitro* by paper disc method. As results showed that among the tested essential oils, Cinnamon oil (82.00 mm), Geranium oil (85.44 mm) and Clove oil (55.00 mm) were completely inhibited the growth of spore germination in *F. oxysporum* while jojoba oil showed the less effective. In *C. capsici* the growth of spore germination was completely inhibited by Cinnammon oil (86.33 mm) and Gerenium oil (87.00 mm). Cinnammon oil, Gerenium oil and Clove oil showed the highest antifungal activity against the both pathogens.

Keywords: Chilli, Fusarium oxysporum, Colletotrichum capsici, essential oils, inhibition.

# Introduction

Chilli (Capsicum annuum L.) is an annual shrub cultivated globally in tropical and sub-tropical areas except colder region. In India chillies were grown all over the part of country and its belonging to family solanaceae. Chillies are vegetables cum spice cum hot testing berry which are economically, commercially and medicinally important crop not only in India but also all over the world. Dry and wet chilli is used in the preparation of various food items i.e. sauces, jams, chocolate, pickles, seasoned, meats, cheeses, vegetables and legumes. Chillies are rich in nutrition like vitamins, especially in vitamin A and C. They are also packed with potassium, magnesium and iron. The medicinal properties are also present in chilli, i.e. they have long been used for pain relief as they are known to inhibit pain messengers, extracts of chilli peppers are used for alleviating the pain of arthritis, headaches, burns and neuralgia.

In India the area use under chilli cultivation is 316.47 thousand hectare and the total production of chilli is 3633.99 thousand MT and among these 44.90 thousand MT of red

spice chilli exported in the world (Gade et al., 2020). India is the largest producer, consumer and exporter country in the world for chilli. Andrapradesh is the leading state in India and in Maharashtra Nandurbar district is stands the first in chilli production. Chilli is the most popular crop in India suffering from many fungal and bacterial diseases and it is the main component to damage the food security in food houses and industries. Some major diseases of chilli that decreases the vield production in many countries viz. Anthracnose, Cercospora leaf spot, Phytophthora, Fusarium wilt, Powdery mildew, Damping-off, Downy mildew and Gray mold. Among these Fusarium wilt and anthracnose are the sever diseases on chilli all over the country and it is caused by Fusarium oxysporum and Colletotrichum capsici respectively. Pre and post harvested yield of chilli losses upto 50% due to the fungal pathogen in many cases in India (Sahitya et al., 2014). Attacking of the anthracnose disease on chilli is a major problem in the marketing quality of capsicum (Kiran et al., 2020). Anthracnose disease damage up to 80% of chilli production in Sleman and Banyumas district (Prihatiningsih

<sup>\*1</sup> Assistant Professor, Department of Botany, Konkan Gyanpeeth Karjat College of Arts, Science & Commerce, Raigad, University of Mumbai, Maharashtra, India.

<sup>&</sup>lt;sup>2</sup>Professor, Department of Botany, K. V. Pendharkar College of Arts, Science and Commerce (Autonomous), Dombivli (E), University of Mumbai, Maharashtra, India.

& Djatmiko, 2020) and 45% in Swamplands of Kalimantan, Indonesia (Mariana *et al.*, 2021). Several species of *Colletotrichum* were caused the anthracnose disease to chilli i.e. *C. acutatum*, *C. gloeosporioides* and *C. capsici*. The symptoms of anthracnose is characterized by circular or angular, depressed, sunken lesions with concentric rings of acervuli on chilli fruit which contains black to white conidial masses.

Fusarium wilt is the second major disease attacking on a chilli crop production in Maharashtra. Wilt is the major disease on pepper crops caused by F. oxysporum in many pepper producing countries (Singh et al., 2017 & Joshi et al., 2012). Different species of Fusarium i.e. F. oxysporum, F. solani, F. moniliforme and F. pallidoroseum have been reported from chilli growing areas as the wilt causing agents but F. oxysporum and F. solani are the most prevalent species of Fusarium associated with wilt of chilli found in India (Naik, 2006). This pathogens can infects more than 100 species of various plants in worldwide and causes the wilt symptoms. Studies of Fusarium wilt in chilli were done by biologically (Nugraheni, 2010), morphologically (Zahara & Harahap, 2007) and enzymatically (Chaiyawat et al. 2008; Wongpia & Lomthaisong 2010). Wilt disease characterized by shrinking the surface of chilli, become dry, white to light brown irregular rings on their surface.

So it is need to control and manage the fungal diseases for increasing the crop productivity and quality. For the same purpose farmers used the excess amount of chemical fertilizers, pesticides and it's harmful and hazardous to environment, human health and microorganism. There is a need to used plant based fungicides, pesticides and fertilizers for integrated disease management in crop cultivation in pre and post harvested diseases. From the ecofriendly plant based product essential oil is one of them. It is a secondary metabolites produced by plant and its recognized antimicrobial and anti-fungal properties (Pandey et al., 2017). It is echo friendly compound obtained from naturally growing plants as compared to the chemical based fungicides which is artificial made by using harmful chemicals. Plant based fungicides are more ecofriendly and less hazardous due to their low toxicity, high degradability and multiple action mechanism than the synthetic fungicides (Deresa & Diriba, 2023). Clove oil has shown the effectively controls on blue mold disease caused by Penicillium italicum incident in citrus (Chen et al., 2019). Potential of biological activity of plant extracts and essential oils has been reported against a wide range of fungal pathogens causes the diseases to plant (Khetabi et al., 2022). Essential oils have their own antimicrobial, anti-oxidant and bio regulatory inbuilt properties. Previously many study reported that, the essential oils are obtained from different plant parts and have indicated fungicidal properties. So that the essential oils is an ideal compound to use as echo friendly pesticides against fungal pathogens. In the present study, we investigated the isolation of F. oxysporum and C. capsici from infected chilli collected from different parts of Maharashtra and tasted the antimicrobial activity of various essential oils against the same fungal pathogens.

### **Materials and Methods**

Collection of Plant Materials: Wilt and anthracnose diseases symptoms showing pre and post harvested chilli collected from different part of Maharashtra and it brought at laboratory in a polythen bags. Infected chilli were kept in 27±2°C room

temperature for 24 hours.

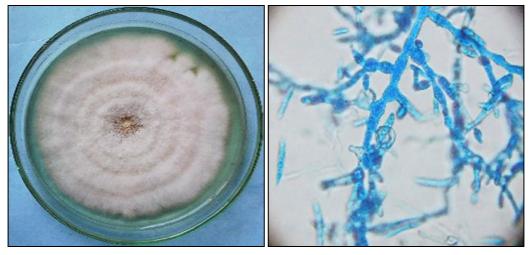
Isolation and Identification: Infected chilli were surface sterilized using silver nitrate (AgNO<sub>3</sub>) for one minute then wash it with sterile/distilled water for excess amount of solver nitrate presents on their surface. Then the infected part of chilli cut into small (5mm) pieces and grown on the plates containing potato dextrose agar (PDA). The plates were incubated at 27±2°C for 2-3 days. A small colonies were appeared in each plates which containing infected part of chilli. Each colony was transferred on a new PDA containing plate and incubated for 7 days to get pure culture of isolate using single spore culture technique. The characterization of both pathogens was done based on their morphology of colonies, cells, macroconidia, microconidia, appresoria and acervuli. Identification of F. oxysporum was done by comparing the morphology described by the atlas of Fusarium (Leslie & Summerell, 2006 and Samson et al., 2008) and the C. capsici was done by comparing the morphology described by Kumar et al. (2015). The pure culture slants of F. oxysporum and C. capsici was prepared and stored into refrigerator for further study.

**Pathogenicity Test:** Seven days old culture of F. oxysporum and C. capsici were used for this test. The mycelium of each pathogens were injected into the healthy surface sterilized chilli with help of forceps using pinprick method. The inoculated chilli was covered with sterile polythen bags and kept for incubation at  $27\pm2^{\circ}C$  room temperature. After some days the symptoms of each disease was slowly developed and it was again re-isolated from the same infected chilli. Both pathogens was compared morphologically and secretion of pigments with the master isolate.

Effect of Essential Oils on Fungal Growth (*In vitro*): Essential oils viz. Eucalyptus oil, Orange oil, Cinnammon oil, Tulsi oil, Camphor oil, Gerenium oil, Nutmeg oil, Motia rosha oil, Lemon oil, Leavendar oil, Clove oil, Rose Mary oil, Jojoba oil, Wheatgerm oil, Walnut oil, Teatree oil, Fenugreek oil, Almond oil, ORPL oil and *Lawsonia inermis* oil were tested individually against biopesticide resistant mutants of *F. oxysporum* and *C. capsici* by using paper disc method. Then the inoculated plates with biopesticide resistant mutant isolates of *F. oxysporum* and *C. capsici* pathogens were incubated at  $27\pm2^{\circ}$ C temperature for 7 days. After seven days, zone of inhibition was recorded of every microorganisms.

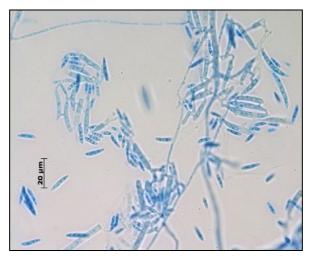
# Result

Targeted fungal pathogens of chilli which caused Fusarium wilt and anthracnose disease were identified on the basis of their meycelial structure, conidia and conidiophores of mycelium. The mycelium of F. oxysporum Schlecht. Emend. Snyder & Hansen was observed with fluffy, white cottony, aerial and in the form of distinct patterns. The colors and pigmentation of Fusarium spp. were to show with white to creamy white, brown and pinkish white on the back side of the PDA containing petri plates. The mycelium of F. oxysporum is septate, branched and its produces macro and micro conidia through conidiophores. Their macro conidia are fusiform, slightly curved, thin walled, pointed at the tip and mostly observed 3-4 septa and microconidia are somewhat hooked apex, never in chain, abundant, mostly non-septate, cylindrical or ellipsoidal, straight or curved and shows the particulate base. Chlamydospores are produces terminally or intercalary (single or in chains). They shows hyaline, smooth and rough walled (Fig. 1).



(a). Pure culture of F. oxysporum.

**(b).** Mycelium with Chlamydospores of *F. oxysporum*.



(c). Macro and Micro conidia of F. oxysporum.

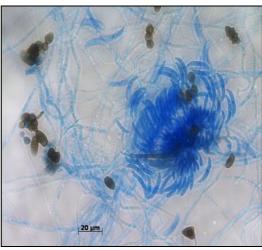
Fig 1: Morphological characteristics of F. oxysporum f. sp. capsici.

C. capsici (Syd.) Butler and Bisby pathogen produces white to gray and dense filamentous mycelium on the PDA containing plate. The mycelium of the C. capsici are septate and branched and it consists of hooked shaped conidia which are produced from acervuli called as subepidermal fruiting body. The conidia usually hyaline, smooth walled, falcate, non-septate, one celled and somewhat curved with pointed at one end. Black colored acervuli was produces with disc shaped and with dark needle like setae from the same fungus.

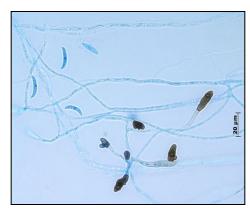
The color of the setae were observed from dark brown to black. On the slide culture appressoria are dark brown, spherical, ovate or obclavate, smooth-walled and supporting hyphae are hyaline, branched, septate, sometimes develop into complex chlamydosporic structures. Both fungal pathogens culture was sent to Division of Mycology, Agharkar Research Institute, Pune, Maharashtra for the purpose of clear images and photographs (Fig. 2).



(a). Pure culture of C. capsici.



**(b).** Mycelium with conidiospores of *C. capsici*.



(c). Mycelium with appressoria of C. capsici

Fig 2: Morphological characteristics C. capsici.

Although essential oils are well known for their antifungal properties but their effectiveness against specific phytopathogenic fungi associated with many plants and crops has not been extensively studied. According to table 1 and 2 twenty essential oils were tested against biopesticide resistant mutant of *F. oxysporum* and *C. capsici*. Among this essential oils Gerenium oil (85.44mm), *Cinnammon* oil (82.00mm) and Clove oil (55.00mm) were showed fruitful result while Tulsi oil, Nutmeg oil, Motia rosha oil, Lemon oil, Teatree oil,

Orange oil, Camphor oil, *Eucalyptus* oil and Rose mary oil were more effective (zone of inhibition in between 10mm to 45mm) against *F. oxysporum* individually and Fenugreek oil, ORPL oil, Walnut oil, Almond oil, Leavender oil, Wheatgerm oil and *Lawsonia inermis* oil were less effective (zone of inhibition in between 5.00mm to 9.9 mm). Jojoba oil was ineffective showing no zone of inhibition was observed (Table 1 and Fig. 3(b)).

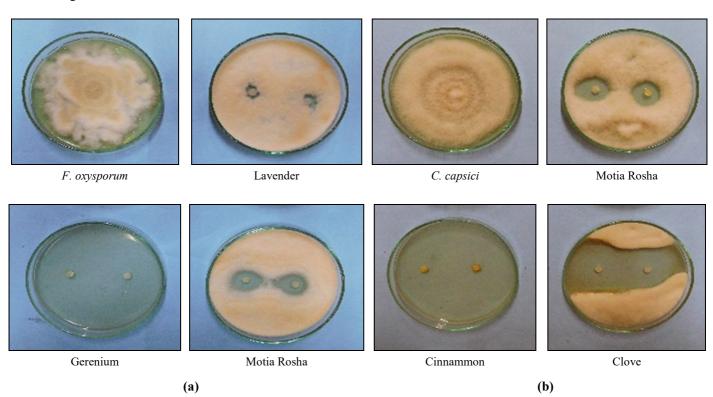


Fig 3: Effect of essential oils against F. oxysporum (a) and C. capsici (b)

Similarly the results of *C. capsici* were depicted in table 2 and fig. 3(b). Out of these essential oils Gerenium oil (87.00mm) and *Cinnammon* oil (86.33mm) gave a fruitful result while Tulsi oil, Clove oil, Motia rosha oil, Nutmeg oil, Lemon oil, Orange oil, Leavendar oil, Fenugreek oil, *Eucalytus* oil, Camphor oil, ORPL oil, Rose Mary oil and Walnut oil were more effective (zone of inhibition in between 10mm to 40mm) and Almond oil, Jojoba oil, *Lawsonia inermis* oil and Wheatgerm oil were less effective (zone of inhibition in between 5.00mm to 9.9mm) against *C. capsici* individually. In the management of *C. capsici* all essential oils were

effective against the same pathogen. The above mentioned essential oils showed a good antifungal activity against both pathogen.

# Discussion

Various essential oils were used in the management of postharvest chilli diseases like Fusarium wilt and anthracnose caused by *F. oxysporum* and *C. capsici*. Total twenty essential oils were collected from ayurvedic medical shop and used against biopesticide resistant

**Table 1:** Effect of essential oils against resistant mutant (*Fo*-EMS-0.01-1) of *F. oxysporum* on PDA.

Su No	Essential Oil	Zone of Inhibition (in Percentage)	
Sr. No.		Mean	% of Inhibition
1	Eucalyptus oil	10.00	88.37
2	Orange Oil	11.33	86.82
3	Cinnammon Oil	82.00	4.65
4	Tulsi Oil	41.33	51.94
5	Camphor Oil	11.67	86.43
6	Gerenium Oil	85.44	0.66
7	Nutmeg Oil	13.67	84.10
8	Motia rosha Oil	13.33	84.50
9	Lemon Oil	13.67	84.10
10	Leavendar Oil	7.67	91.08
11	Clove Oil	55.00	36.04
12	Rose Mary Oil	10.67	87.59
13	Jojoba Oil	0.00	100.00
14	Wheatgerm Oil	6.33	92.63
15	Walnut Oil	9.33	89.15
16	Teatree Oil	13.00	84.88
17	Fenugreek Oil	9.00	89.53
18	Almond Oil	8.67	89.91
19	ORPL Oil	9.00	89.53
20	Lawsonia inermis Oil	5.67	93.40
21	Control	86.00	
	SE	6.127563	6.30318094
	CD at 0.05%	12.781965	13.148266
	0.01%	17.432737	17.932319

<sup>\*</sup> Value are the mean of three replicates.

**Table 2:** Effect of essential oils against resistant mutant (*Cc*-EMS-0.01-2) of *C. capsici* on PDA.

Sr. No.	<b>Essential Oil</b>	Zone of Inhibition (in percentage)	
		Mean	% of Inhibition
1	Eucalyptus oil	12.00	86.20
2	Orange Oil	17.00	80.45
3	Cinnammon Oil	86.33	0.77
4	Tulsi Oil	38.33	55.94
5	Camphor Oil	12.67	85.43
6	Gerenium Oil	87.00	0.00
7	Nutmeg Oil	18.00	79.31
8	Motia rosha Oil	19.33	77.78
9	Lemon Oil	17.33	80.08
10	Leavendar Oil	13.67	84.28
11	Clove Oil	37.33	57.09
12	Rose Mary Oil	10.33	88.12
13	Jojoba Oil	7.67	91.18
14	Wheatgerm Oil	5.67	93.48
15	Walnut Oil	10.00	88.50
16	Teatree Oil	11.67	86.58
17	Fenugreek Oil	13.67	84.28
18	Almond Oil	9.33	89.27
19	ORPL Oil	12.00	86.20
20	Lawsonia inermis Oil	6.67	92.33
21	Control	87.00	
	SE	5.97186	5.82108
	CD at 0.05%	12.45717	12.14261
	0.01%	16.98977	16.56075

<sup>\*</sup> Value are the mean of three replicates.

Mutant of *F. oxysporum* and *C. capsici* pathogens to check their antifungal activity against them. Out of these twenty essential oils viz. Gerenium oil, Cinnammon oil, Clove oil, Tulsi oil, Nutmeg oil, Motia rosha oil, Lemon oil, Teatree oil, Orange oil, Camphor oil, Eucalyptus oil and Rose mary oil showed the zone of inhibition (10mm to 45mm) fruitfully against *F. oxysporum* individually while Fenugreek oil, ORPL oil, Walnut oil, Almond oil, Leavender oil, Wheatgerm oil and *Lawsonia inermis* oil showed effectiveness zone of inhibition against tested fungi.

Like as a *F. oxysporum* in *C. capsici* Gerenium oil, Cinnammon oil, Tulsi oil, Clove oil, Motia rosha oil, Nutmeg oil, Lemon oil, Orange oil, Leavendar oil, Fenugreek oil, Eucalyptus oil, Camphor oil, ORPL oil, Rose mary oil and Walnut oil showed the zone of inhibition (10mm to 40mm) fruitfully against *C. capsici* individually while least zone of inhibition was showed by almond oil, Jojoba oil, *Lawsonia inermis* oil and Wheatgerm oil against tested pathogen. Almost tested all essential oils were to show more or less antifungal activity against tested *F. oxysproum* and *C. capsici* pathogen.

Similar results were observed by, Yulia *et al.* (2006) observed Cinnamon bark and leaf oils completely inhibited the mycelial growth of *Colletotrichum* at the concentrations equal to or greater than 0.1% and Cardamom oil completely inhibited the germination at concentrations greater or equal to 1% while Clove bud and leaf essential oils were less effective than Cinnamon and Cardamom oil. Istianto and Emilda (2011) evaluated that all the tested essential oils showed that they were able to suppress *Foc* mycelial growth. Mainly the extracted essential oil from *E. aromatic* provided the strongest suppression of *Foc* mycelial growth at a volume of 9 and 18µl.

Begum and Nath (2015) were noted that Garlic gave 100% inhibition of a mycelial growth of all the four isolates (Scc<sub>1</sub>, Scc<sub>2</sub>, Scc<sub>3</sub> and Scc<sub>4</sub>) of *C. capsici* at all the concentrations tested. However neem oil showed the 100% inhibition of mycelial growth of all *C. capsici* isolates at 0.1% and 0.2% concentration. Citronella oil was showed least effective among the botanicals oil. Duduk *et al.* (2015) suggested from their antifungal activity work, that thyme and cinnamon bark essential oils was effective against *C. acutatum* both *in vitro* and *in vivo* conditions.

Jagtap and Suryawanshi (2016) revealed that Clove oil showed a maximum zone of inhibition against *Fusarium oxysporum* when applied individually (42mm) and in mixture with thiophanate methyl (90mm) followed by Geranium, Motiarosha, Tulsi, *Eucalyptus*, Rosewood and *Cinnamon* oil. Singh *et al.* (2017) reported the antifungal activity of neem oil, garlic oil and mustard oil at 1, 2, 5 & 10% concentrations was tested against *Fusarium oxysporum* causing wilt of chilli. The neem oil (Nemazal) and garlic oil at 10% concentration completely inhibited the mycelial growth that was followed by mustard oil (69.26%).

Balendres and Fm (2019) found that the mycelial growth of all tested fungal pathogens was strongly inhibited by Citronella essential oil (CEO), but variations were observed among fungal species. So their glasshouse and field experiments would established CEO as one of the potential alternative to chemical fungicide. Mekonnen *et al.* (2019) found that Lemon grass and Spear mint essential oils showed the maximum antifungal activity against *Fusarium* wilt whereas Eucalyptus essential oil inhibit the list amount of mycelial growth of tested pathogen.

De Castro et al. (2020) reported that the essential oil of V.

curassavica provided greater mycelial growth inhibition at different concentrations of 0.05, 0.1, 0.5, 1.0 & 3.0% (v/v) in PDA medium of *C. musae* when it was compared with other essential oil by using both *in vitro* and *in vivo* conditions. Samithri *et al.* (2020) investigated that citronella oil was the most effective as compared with other essential oils. Cardamom and Citronella oil significantly inhibited the mycelial growth of *Colletotrichum* sp. compared with other essential oils. While lemon, orange and mustard oils did not cause significant inhibition of mycelial growth.

Gairhe *et al.* (2021) evaluated that at 1000ppm concentration, Cinnamon oil shows the lowest mycelial growth and highest percent growth inhibition (1.67mm and 98.15%) followed by mustard oil (54.00mm and 40.00%), neem oil (55.17mm and 38.70%), castor oil (55.83mm and 37.96%), coconut oil (61.17mm and 32.04%) as compared to control (90mm and 0.00%) respectively. Hodiyah *et al.* (2022) were evaluated the effect of clove oil in inhibiting the growth of three major causal agent of pepper diseases namely *Colletotrichum acutatum, Phythopthora capsici* and *Pythium* sp. through *in vitro* technique by using different concentrations of essential oil. The best inhibitory effect of *Syzygium aromatium* L. essential oil was showed at the concentration of 340μl/L, 180μl/L and 100μl/L for *C. acutatum, P. capsici* and *Pythium* sp. respectively.

Hodiyah *et al.* (2023) suggested that *Cymbopogon citratus* L. essential oil continuously showed a reduction in the mycelial colony development of *Colletotrichum* sp. consistently until 6 DAI, 44.38% & 86.82% for *P. capsici. Syzygium aromaticum* essential oils showed 100% inhibition against *P. capsici* and 91.71% for *Colletotrichum* sp. for their mycelium development on chili (*Capsicum annum* L.). Yuan *et al.* (2024) reported that rosemary essential oil was able to inhibit the growth of *C. gloeosporioides*, with minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values of 15.625  $\mu$ L/mL and 31.25  $\mu$ L/mL, respectively.

Medina and Ruales (2025) evaluated that essential oils of oregano (*Origanum vulgare*) and cinnamon (*Cinnamomum verum*) demonstrated effective inhibition of fungal growth at a concentration of 600 ppm. The findings of this study indicate that essential oils like oregano, cinnamon, and clove are effective natural alternatives for managing postharvest diseases in bananas.

### Conclusion

Essential oils provide an environmentally sustainable and often effective method for managing wilt and anthracnose diseases in chilli plants. While they may not entirely replace current traditional chemical treatments but their use as a part of an integrated pest management strategy. When it combined with regular application and proper dilution then it can be significantly reduce disease incidence and promote healthier crops.

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