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EFFICACY OF ESSENTIAL OIL OF ZINGIBER OFFICINALE (ROSC) IN PRESERVATION OF ORANGE (CITRUS SINENSIS (L.)OSBEC FRUIT AGAINST ROTTING FUNGI

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ABSTRACT

The antifungal activity of essential oil of *Zingiber officinale* was tested agianst the post harvest fungal species of oranges viz., *Penicillium italicum*, *P. digitatum*, *Geotrichum citri*, *Alternaria alternata*, *Colletotrichum gloeosporioides* and *Phomopsis citri*. The oil was standardized through physicho-chemical and fungitoxic properties. The yield of oil was 1.2% .The specific gravity of the oil was 0.890. The saponification value was 23.84. The ester value was 19.36.The acid value was 4.48.The MIC of the oil was 100ppm against *P. italicum* and *P.digitatum*, 200ppm against *G. citri* and 500ppm against *A.alternata*, *C.gloeosporioides* and *P.citri*. The nature of toxicity of the oil was found to be static at the respective MICs of the oil against different fungi. At higher concentration the nature of toxicity of the oil was turned to cidal in nature. The oil was found to be thermostable up to 80° C of temperature.The oil retained its fungitoxicity up to 2 years. The fungitoxicity of the oil tolerated the higher inoculums densities of all the tested post harvest fungi. The oil was found to be more efficacious than some prevalent synthetic fungicides on the basis of having lower MIC as compared to synthetic ones.

INTRODUCTION

Citrus is one of the most widely produced fruit globally. The importance of citrus fruit is attributed to its diversified use, which is widely consumed either as fresh fruit or as juice. Due to their higher water content, high levels of sugars and nutrients, citrus fruits are very susceptible to infection by microbial pathogens during the period between harvest and consumption (Tripathi and Dubey, 2003; Singh and Sharma, 2007). Citrus fruits are usually acidic in nature and pH range between 2.2 and 4. Due to this, the most decay after harvest of citrus fruits is caused by fungi. Contamination and infection by pathogenic fungi occur at different stages in the field and after harvest and usually follows mechanical injury of the fruit, which allows entry of these micro-organisms. Postharvest decays of fruit can also originate from latent infections occurring in the orchard such as black rot caused by *Alternaria alternata* pv. citri, brown rot caused by *Phytophthora citrophthora* and anthracnose caused by *Colletotrichum gloeosporioides*. In developing and undeveloped countries, high losses result from inadequate storage facilities and improper transport and handling (Ladaniya, 2008).

Citrus fruits are susceptible to a number of postharvest diseases that cause significant losses during the postharvest phase. Nevertheless, the most common and serious diseases that affect citrus fruit are green and blue moulds caused, respectively, by *Penicillium digitatum* Sacc. and *Penicillium italicum* Wehmer, followed in importance by sour rot caused by *Geotrichum citri-aurantii* Link ex Persn (Caccioni *et al*. 1998; Palou *et al*. 2002; Zheng *et al*. 2005). These pathogens are strict wound pathogens that can infect the fruit in the grove, in the packinghouse, or during subsequent handling and storage (Palou *et al.* 2008). The fungal inoculum is practically always present on the surface of fruit during the season and after harvest can build up to high levels unless appropriate packinghouse sanitization measures are adopted (Kanetis *et al.* 2007). Fruit infection by these fungi is enhanced during the fruit degreening operation and during wet and rainfall seasons (Liu *et al*. 2009). Decay is also more prevalent as fruit increases in maturity, and at favourable temperatures and humidity (Baudoin and Eckert 1985).

Over the years, the protection of agricultural crops and products was achieved almost entirely through the use of synthetic chemicals. These chemicals, though valued for their effectiveness in controlling various post harvest diseases of fruits, are costly and their continued or repeated applications may disrupt equilibrium of ecosystems, leading to dramatic disease outbreaks, widespread development of pathogens resistant to one or more chemicals, toxicity to non-target organisms and environmental problems. Sometimes, they accumulate in the food chain as residues. Furthermore, pesticide residues in food possess more carcinogenic risks than insecticides and herbicides (Lee *et al.* 2009). These highlighted

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the need to develop alternative control strategies or innovative crop protection and postharvest methods of fruit rot control with a reduced use of conventional fungicides or without synthetic chemicals (Kim *et al*. 2003).

Biologically active essential oils represent a rich potential source of an alternative and perhaps environmentally more acceptable disease management compounds. With a broad range of natural fungicidal plant volatiles, numerous opportunities exist to explore their usefulness in controlling post-harvest diseases. The general antifungal activity of essential oils is well documented (Pitarokili *et al*. 1999; Meepagala *et al*. 2002) and there have been some studies on the effects of these oils on post-harvest pathogens (Bishop and Thornton, 1997). The advantage of essential oils is their bioactivity in the vapour phase, a characteristic which makes them attractive as possible fumigants for stored product protection. Therefore, it seemed opportune to assay the *in vitro* fungicidal activity of essential oil of *Zingiber officinale* (Rosc) (Family- Zingiberaceae) against six well known postharvest fruit decaying agents of citrus fruits. The *Z. officinale* has been in use in traditional medicine since long and its fungitoxic products would be having nonmammalian toxicity if applied in the management of storage fungi of fruits.

MATERIALS AND METHODS

Isolation of volatile fungitoxic fraction (essential oil) from the rhizomes of Z.officinale

Since the rhizomes of *Zingiber officinale* were having strong aromatic odor, the rhizomes were selected for the isolation of essential oil. 250 g of fresh rhizomes were cut into small pieces and then thoroughly washed with sterilized water. The essential oil was isolated by Clevenger's apparatus. The isolated fraction showed two distinct layers-an upper oily layer and a lower aqueous layer. Both the layers were separated and the moisture from the oily layer was removed by adding anhydrous sodium sulphate.

Isolation of fungal pathogen from orange fruit (Citrus sinensis **(L.) Osbeck)**

Fungal pathogens were isolated from infected orange fruits. The diseased parts were cut into small pieces (2-3mm) and surfaced sterilized with 0.1% mercuric chloride solution for 30 seconds. The pieces were then washed three times with sterilized distilled water and aseptically transferred on to clean sterilized Petridishes containing solidified potato dextrose agar medium. The Petridishes were incubated in inverted position at 28±1°C and observed after 3-4 days. Fungal hyphae, growing out from the infected fruit pieces associated with post harvest disease of oranges were identified microscopically (Burnett and Hunter 1999) and purified on PDA slants. Pure culture was maintained by periodic sub culturing (Aneja 2004).

The fungal cultures of *Botrytis cinerea* (Pers), *Botryodiplodia theobromae* (Pat), *Ceratocystis paradoxa* (Dade) C. Moreau, *Fusarium roseum* (Link), *Monilinia fructicola* (G Winter) Honey*, Macrophomina phaseolina* (Tossi) Goid and *Penicillium expansum* (Link) were procured from IARI New Delhi.

Pathogenicity test

The pathogens were isolated, identified and cultures were used to confirm pathogenicity on orange fruits. The fruits were washed with distilled water and allowed to dry under a fan and then surface sterilized with 0.1% mercuric chloride solution. The Wounds were made in the fruit with the help of sterilized cork borer (2mm). The wounds were inoculated with pathogen containing spore load of $1x10^4$ conidia/ml (Granger and Horne 1924). The inoculated fruits were wrapped in sterilized paper and incubated at 28±1°C. The artificially inoculated fruits were examined daily and extent of damage was recorded. The pathogens were re-isolated and disease symptoms were clearly evident, the culture and symptom were compared with original.

Antifungal testing and Minimum inhibitory concentration (MIC) of essential oil of Z. officinale

Fungitoxicity of the extracted oil was tested by the poisoned food technique (Pandey *et al.,* 1982, Perrucci *et al.* 1994) by using Czepeks agar medium. The concentration of the essential oil was prepared by dissolving the requisite amounts in 0.5ml of 0.1% Tween-80 and then mixing with 9.5 ml of Czepeks agar medium to produce 600ppm, 500ppm, 400ppm, 300ppm, 200ppm, 100ppm and 50ppm concentrations. The control sets were prepared similarly using equal amounts of sterilized distilled water in place of oil.

Nature of toxicity of the essential oil of Z.officinale

The nature of toxicity (fungitoxic/ fungicidal) of the essential oil was tested against the test fungi following Thompson (1989).The inhibited fungal discs of the oil treated sets were re-inoculated in to fresh medium and revival of their growth was observed.

Range of fungitoxicity of the essential oil of Z.officinale

The range of fungitoxicity of essential oil of *Z. officinale* was tested against 10 most common fruit and vegetables rotting fungi viz*., Aspergillus niger, Botrytis cinerea*, *Botriodiplodia theobromae, Ceratocystis paradoxa*, *Fusarium roseum, Monilinia fructicola*, *Mucor piriformis, Macrophomina phaseolina*, *Penicillium expansum*,and *Rhizopus stolonifer* by poison food technique.

The effect of increased inoculum density of the tested fungi on fungitoxicity of the essential oil of Z.officinale

The effect of increased inoculum density of the tested fungi (viz. *Penicillium italicum, P. digitatum, G. citri-aurantii, B. theobromae, C. Gleosporioides and P. citri*) on fungitoxicity of the *Z. officinale* oil was studied following Moleyar and Pattisapu (1987). Requisite quantity of the oil was dissolved in 0.5 ml of 0.01 per cent aqueous solution of Tween -80 and then mixed to 9.5 ml liquid potato dextrose medium to make the final concentration of 500ppm.Seven sets for each fungus were thus prepared and were inoculated separately by the assay discs (5mm in dia.) of test fungi separately in geometric progression of two i.e.,1,2,4,8,16,32,and 64.The approximate number of spores of each set was recorded with the help of heamocytometer by introducing the similar number of fungal discs in distilled water. For control set equal quantity of sterilized water dissolved in 0.5 ml Tween -80 was mixed with the liquid potato dextrose medium. All the sets prepared separately for different fungi (viz. *Penicillium italicum, P. digitatum, G. indicum, B. theobromae, C. Gleosporioides and P. citri*) were incubated for six days at 27±1˚C and observations were recorded on the seventh day.

Effect of storage and temperature on fungitoxicity of essential oil of Z.officinale

To determine the effect of storage, the oil stored in air tight specimen tubes at room temperature was subjected to fungitoxic testing at its MIC at a regular interval of one month by the usual poisoned food technique.

To study the effect of temperature, five lots of oil each containing 2ml of the oil, were kept separately in air tight glass vials and were kept at different temperatures viz., 5˚C,10˚C,40˚C,60˚C and 80˚C for three hours. The oil of each tube was tested for fungitoxicity at its MIC by the usual poisoned food technique and percentage inhibition of growth of test fungi was recorded.

Physicochemical properties of the essential oil of Z.officinale

The oil was standardized for physic-chemical properties viz. specific gravity, optical rotation, refractive index, solubility in different organic solvents, acid number, saponification value, ester value and phenolic content following Chawdhury and Kapoor (2000).

Comparison of the fungitoxicity of the essential of Z.officinale with some prevalent synthetic fungicides

The efficacy of the oils was compared with some fungicides, viz. benzimidazole (benomyl), diphenylamine, phenylmercuric acetate (ceresan) and zinc dimethyl dithiocarbamete (zirum) by the usual poisoned food technique.

RESULTS

Volatile fungitoxic fraction from Z.officinale rhizome

The volatile fungitoxic fraction was obtained in the form of yellow colored essential oil with pleasant aromatic odour. The recovery of the oil from the *Z. officinale* rhizomes was 1.2%.

The isolated fungal pathogens and their pathogenicity test

During the isolation of fungal pathogens from the infected fruits of oranges six fungal pathogens were isolated. These were *Penicillium italicum* Wehmer, *P. digitatum* (Pers.) Sacc, *Geotrichum citri-aurantii* Ferraris.Butler, *Alternaria alternata* (Fr.) Keissi, *Colletotrichum gloeosporiodes* (Penz.) Penz.Sacc and *Phomopsis citri* (Fawc). The isolated pathogens were found to show the same specific characters of symptoms associated with them during pathogenicity test.

Minimum Inhibitory Concentration of the essential oil of Z.officinale

The oil completely inhibited mycelial growth of the *P. digitatum* and *P. italicum* at 100ppm, *G. indicum* at 200ppm and *A. alternata, C. gloeosporioides* and *P. Citri* at 500ppm.

Table 1 Minimum inhibitory concentration of the *Z. officinale* essential oil against the pathogens

Concentration (ppm)	Percent Inhibition in growth of test fungi					
	P.italicum	*P. digitatum*	*G.indicum*	*A.alternata*	*C.gleosporioides*	*P.citri*
50	60.00	70.00	30.00	10.00	5.00	15.00
100	100.00	100.00	70.00	25.00	25.00	25.00
200	100.00	100.00	100.00	55.00	45.00	40.00
300	100.00	100.00	100.00	70.00	80.00	70.00
400	100.00	100.00	100.00	80.00	90.00	95.00
500	100.00	100.00	100.00	100.00	100.00	100.00
600	100.00	100.00	100.00	100.00	100.00	100.00

Table2 Nature of toxicity of Essential oil of *Z. officinale* against test fungi

Concentration (ppm)	P.italicum	P. digitatum	G.indicum	A.alternata	C.gleosporioides	P.citri
100	static	static				
200	cidal	cidal	static			
300			cidal			
400						
500				Static	static	static
600				Cidal	cidal	cidal

Table 3 Effect of increased inoculum density of test fungi on the fungitoxicity of the oil

Number of fungal discs	Approximate number of spores	Growth of test fungi											
		P.italicum		*P.digitatum*		*G.indicum*		*A.alternata*		*C.gleosporioides*		*P.citri*	
1	2358X10^{3}	T	C	T	C	T	C	T	C	T	C	T	C
2	47174X10^{3}	-	+	-	+	-	+	-	+	-	+	-	+
4	94348X10^{3}	-	+	-	+	-	+	-	+	-	+	-	+
8	188696X10^{3}	-	+	-	+	-	+	-	+	-	+	-	+
16	377392X10^{3}	-	+	-	+	-	+	-	+	-	+	-	+
32	754784X10^{3}	-	+	-	+	-	+	-	+	-	+	-	+
64	1509568X10^{3}	-	+	-	+	-	+	-	+	-	+	-	+

T= Trattment, C=Control, (-) indicates no growth of test fungus, (+) indicates growth of test fungus

It indicated that the MIC of the oil to be 100 ppm against *P. digitatum* and *P. italicum*, 200ppm against *G. indicum* and 500ppm against *A. alternata,C. gloeosporioides* and *P. citri* (Table-1).

Nature of toxicity of the essential oil of Z.officinale

The fungitoxicity of the essential oil was found to be static in nature at their respective MIC against different fungi. The static nature of the oil was turned cidal against *P. digitatum* and *P. italicum* at 200ppm. At 300ppm the toxicity of the oil was found to be cidal against *G.indicum*. At 600ppm the static nature of the oil was turned cidal against *A.alternata*, *C. gloeosporioides* and *P. citri* (Table-2).

Fungitoxic properties of the oil

It has been observed that the oil inhibited the fungal growth of the treatment sets containing even 64 discs of the all the tested fungi indicating the potency of the oil to withstand high inoculum density (Table-3). It was found that the oil had long shelf life. The oil remained active up to two years. The oil was thermostable in nature as it remained fungitoxic at different temperatures between 5˚C and 80 ºC.

Range of fungitoxicity of th essential oil of Z.officinale against other fruit and vegetable rotting fungi

The essential oil of *Z. officinale* exhibited a broad range of fungitoxicity inhibiting growth of all the 10 most common fruit and vegetable rotting fungi viz. *Aspergillus niger, Botrytis cinerea*, *Botriodiplodia theobroma,e Ceratocystis paradoxa*, *Fusarium roseum, Monilinia fructicola*, *Mucor piriformis* and *Macrophomina phaseolina, P. expansum*, and *Rhizopus stolonifer* (Table-4).

Table 4 Fungitoxic spectrum of *Z.officinale* oil at 500ppm

Fungi tested	Per cent inhibition of growth of test fungi
Aspergillus niger Van Teigh	100
Botrytis cineria (Pers)	100
Botryodiplodia theobromae Pat	100
Ceratocystis paradoxa (Dade) C. Moreau	100
Fusarium roseum (Link)	100
Monilinia fructicola (G.Winter) Honey	100
Mucor piriformis Scop	100
Macrophomina phaseolina (Tossi) Goid	100
Rhizopus stolonifer	100
Penicillium expensum (Link)	100

Physico-chemical properties of the oil

The various physicho-chemical properties viz. specific gravity, specific rotation, refractive index, solubility in different organic solvents, saponification value, ester value and phenolic content of the oil are recorded in (Table-5).

Comparative efficacy of oil

MIC of synthetic fungicides benzimidazole, diphenylamine, phenylmercuric acetate and zincdimethyl dithiocarbamate was found to be 300, 800, 600 and 700ppm respectively which was higher as compared to *Zingiber officinale* oil (100 ppm against *P. digitatum* and *P. italicum*, 200ppm against *G. citri-aurantii*, 500ppm against *A. alternata, C. gloeosporioides and P. citri*). Therefore the oil was more potent than the synthetic ones.

Table5 Physico-chemical properties of essential oil of *Zingiber officinale*

Parameters	*Z. officinale* essential oil
Yield of oil	1.2%
Colour	Yellow
Odour	Aromatic pleasant
Spesific gravity	0.8900
Optical rotation	(-)5˚
Refractive index	1.4840 (at 24˚C)
Solubility	
Acetone	Soluble (1:1Conc)
Absolute alcohol	Soluble (1:1Conc)
90%alcohol	Soluble (1:1Conc)
Ethyl acetate	Soluble (1:1Conc)
Benzene	Soluble (1:1Conc)
Chloroform	Soluble (1:1Conc)
Hexane	Soluble (1:1Conc)
Methanol	Soluble (1:1Conc)
Acid number	4.48
Saponification value	23.84
Ester value	19.36
Phenolic content	Present

DISCUSSION

Investigations on the success of essential oils as biodegradable and ecofriendly fungitoxicants have shown the possibilities for their exploitation as natural fungicides (Dixit *et al*. 1995, Tripathi *et al*. 2004). The fungicidal activities of essential oils of Ocimum, Thymus, Origanum, Anethum, Eucalyptus, Foeniculum and Citrus have been reported against post harvest pathogens (Caccioni and Gizzardi 1994, Gujar and Talwankar 2012).

In the present investigation the volatile fungitoxic principle of *Z. officinale* was obtained in the form of essential oil. The recovery of the essential oil of *Z. officinale* depends on variety and origin of the plant as well as the cultivation, humidity at the time of harvest, the methods of extraction and to some extent on the age of the plant (Onyenekwe and Hashimoto 1999). We found the yield of oil as 1.2% of the total weight. The oil was yellow in color with aromatic and pleasant smell.

It is essential to recommend the minimum dose in comparison of the pesticides. Therefore the minimum inhibitory concentration was determined. The minimum inhibitory concentration of *Z. officinale* oil was found to be varied against the different tested fungi. It was100ppm against *P. italicum* and *P. digitatum*. The MIC of the oil was 200ppm against the *G.citri-aurantii*. Similarly against *A. alternata, C. gloeosporooides* and *P. citri* the MIC of oil was 500ppm. On the basis of these findings we can say that the MIC of oil varied according to the test pathogens. It was highest (500ppm) against the stronger pathogens (*A .alternata*, *C goloeodides* and *P.citri*). The minimum MIC of 100ppm was observed with milder pathogens i.e. *P. italicum* and *P. digitatum*. Fatemi *et al*. (2011) also reported the MIC of *Thymus vulgaris* and *Saturega hortensis e*ssential oil to be 200 and 400ppm respectively.

The fungitoxicity of the essential oil was found to be static in nature at their respective MIC against different fungi. The static nature of the oil was turned cidal against *P. digitatum* and *P. italicum* at 200ppm. At 300ppm the toxicity of the oil was found to be cidal against *G. citri-aurantii*. At 600ppm the static nature of the oil was turned cidal against *A. alternata*, *C. gloeosporioides* and *P.citri.*

The oil was found to withstand high inoculum density as the oil has shown the antifungal activity in the treatment sets of all the tested fruit rotting fungi (viz. *P. italicum, P. digitatum, G. citri-aurantii, A. alternata, C. gloeosporiidesand P. citri*) containing the 64 fungal discs. This is another potential of the oil to be exploited as botanical fumigant. The oil remained fungitoxic for up to 2 years having long shelf life. The oil was thermostable in nature as it can withstand up to 80ºC temperature without losing toxicity. The oil exhibited a wide range of activity at 500 ppm against 10 most common fruit and vegetables rotting fungi. Namely *Aspergillus niger, Botrytis cinerea*, *Botriodiplodia theobromae, Ceratocystis paradoxa*, *Fusarium roseum, Monilinia fructicola*, *Mucor piriformis* and *Macrophomina phaseolina, P. expansum*, and *Rhizopus stolonifer.*

On comparing the MIC of the oil with some synthetic fungicides the oil was more active than the synthetic pesticides as the MIC of the oil was found to be low as compared to synthetic fungicides. MIC of synthetic fungicides benzimidazole, diphenylamine, phenylmercuric acetate and zincdimethyl dithiocarbamate was found to be 300, 800, 600 and 700ppm respectively which was higher as compared to *Zingiber officinale* oil (100 ppm against *P. digitatum* and *P. italicum*, 200ppm against *G. candidum*, 500ppm against *A. alternata, C. gloeosporioides and P. citri*). Therefore the oil was more potent than the synthetic ones.

The essential oil and oleoresin of *Z. officinale* are used as a medicine with indications against several problems, such as a cure for swelling, sores and loss of appetite (Nhareetsomchit and Nurshukriyah 2003). Some of these functional properties are generally attributed to the gingerol and shogaol. For instance, gingerols inhibited the growth of *Mycobacterium avium* and *Mycobacterium tuberculosis* (Hiserodt *et al*. 1998). The essential oil of *Z. Officinale* has shown moderate to good inhibitory effects against some of fungi such as *Aspergillus flavus*, *Aspergillus solani*, *Aspergillus oryzae, Aspergillus niger* and *Fusarium moniliforme* (Gurdip Sing *et al.* 2008). At present, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain cases. Fortunately, even long term consumption of these substances is not known to produce any side effects. In recent years, in view of their beneficial effects, use of ginger and many other spices /herbs as medicine has been gradually increasing in many countries (Langer 1998). Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted the attention in recent years. Rhizome of *Z. officinale* has been recommended for use as carminative, diaphoretic, anti spasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti inflammatory agent, diuretic and digestive aid (Langner *et al*. 1998, White 2007). Research works have been carried out concerning the effects of *Z. officinale* in surgery, chemotherapy and some other illness (Schmid *et al*. 1994, Visnovsky 1992, Bliddal *et al*. 2000).

Therefore, the essential oil of Z.officinale with low MIC in comparison to synthetic ones, fungicidal nature against the test fungi as well as against other common fruit rotting fungi, thermostable nature and the efficacy to withstand high inoculum density are the important characters that the oil of *Z.officale* has shown in the present investigation. These attributes are quite sufficient to recommend it as a safe fungitoxicant. It has the potential to be used as botanical fungicidal fumigant. Some other workers have shown the activity of some other essential oils against the post harvest fungi of citrus fruits. Plaza *et al*. (2004) also reported that thyme and cinnamon essential oils significantly reduced the incidence of green and blue moulds of citrus. Also, thyme oil was reported to control most postharvest citrus rots, such as green mould, blue mould and sour rot (Arras and Usai 2001, Liu *et al*. 2009). The antifungal activity of the essential oils suggests that they may be considered as a potential alternative to the synthetic fungicides for the control of postharvest citrus pathogens

Therefore, use of these substances as antimicrobial agents can be an interesting field of investigation as the toxicity to mammals is quite low, and their degree of volatility allows their use for fumigation in cold storage or for active packaging. However, the investigations on the mode of action and practical applicability of such plant products is still required so as to recommend their formulation in control of postharvest diseases. However, despite their potent antifungal activity, commercial implementation of treatments with essential oils is strongly restricted in citrus because of problems related to potential phytotoxicity, intense sensory attributes or technological application as fumigants or in aqueous solutions (Palou *et al*. 2008).

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