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Biorational Management of Post-harvest Sour Rot of Kinnow Fruits

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Abstract

The experiment was conducted during the year 2007 at the Agricultural Research Station, Sriganganagar (Raj.). Kinnow (*Citrus deliciosa*) fruits were prone to attack by several microorganisms during storage and marketing. Sour rot caused by *Geotrichum candidum* Link. was one of the most prominent post-harvest rots of Kinnow fruits. Twelve native and exotic strains of fungal [*Trichoderma hamatum* (HP-20), *T. harzianum* (TG-1), *T. viride*-1, *T. viride*-2, *Gliocladium deliquescens, G. virens,* and *Chaetomium globosum* (HP-29)], yeasts [*Debaryomyces hansenii, Sporidiobolus pararoseus* (KFY-1)], and bacterial [*Bacillus subtilis, Pseudomonas fluorescens* (CHAO), *P. fluorescens* (Pf-1)] antagonists were evaluated against the *G. candidum*. *Debaryomyces hansenii* and *S. pararoseus* (KFY-1) proved superior over the rest of the antagonists tested against the pathogen by inhibiting 91.67 and 87.22% mycelial growth, respectively. *Pseudomonas fluorescens* (Pf-1), *B. subtilis,* and *P. fluorescens* (CHAO) exhibited 77.04, 69.07, and 66.67% growth inhibition, respectively. Maximum reduction in rot incidence was observed in fruits treated with spore suspension (10^9 cfu ml^{-1}) of *D. hansenii* and *S. pararoseus* (KFY-1), where 88.30 and 84.11% in pre-inoculation and 85.32 and 78.79% in post-inoculation treatment were noted, respectively. Spore suspension (10^9 cfu ml^{-1}) of *P. fluorescens* (Pf-1) and *B. subtilis* was found to be the next in order of bioefficacy against the fruit rotting, protected the treated fruits to the extent of 79.94, 77.15 and 74.18, 69.84%, respectively, in pre- and post-inoculation treatments.

Keywords: Post-harvest sour rot, kinnow, *Geotrichum candidum*, antagonists

1. Introduction

Kinnow (*Citrus deliciosa* Ten.) is a hybrid variety of the mandarin group of citruses (Mahawar et al., 2019). It is a hybrid of two citrus cultivars, namely 'King' (*Citrus nobilis*) and 'Willow Leaf' mandarin (*Citrus deliciosa*). In India, Mandarins were grown at 4.3 lac ha area with production and productivity of 49.82 lac tonnes and 11.59 tonnes ha^{-1}, respectively (Anonymous, 2018). Kinnow fruits were affected by several post-harvest rotting. The aggregate post-harvest losses in Kinnow, from orchard to consumers, ranged from 14.87 to 21.91% (Gangwar et al., 2007). Sour rot of Kinnow fruits incited by *Geotrichum candidum* Link. was one of the most destructive diseases, causing nearly 14% of the total post-harvest fruit decay (Sharma et al., 2010). Post-harvest rots management in citrus fruits relies mainly on the use of fungicides, but because of the emergence of strains of pathogens resistant to fungicides (Suhr and Nielsen, 2003) and the growing concern for human safety and the protection of the environment, an attempt was made to search for alternatives to the use of synthetic fungicides for the management of post-harvest rots. Biorational management through antagonistic microorganisms has emerged as an effective strategy to combat major postharvest fungal rotting of fruits and forms a realistic substitute to chemical fungicides. Hence, an attempt has been made to evaluate effective bioagents (fungal, yeast, and bacterial) against the *Geotrichum candidum* under *in vitro* conditions and further explore the bioefficacy of potential bioagents in controlling the incited post-harvest sour rot of Kinnow fruits.

2. Materials and Methods

The experiment was conducted during the year 2007 at Agricultural Research Station, Sriganganagar (Raj.)

2.1. Pathogenicity test of the isolated fungus and identification of the rot

The pathogenicity was proved by inoculating the injured healthy fruits with standard spore suspension (1×10^6 spores ml^{-1}) of the fungus, *G. candidum* isolated from rotted fruits, collected during the survey of retail fruit markets and cold storages. Fruits with pedicels were injured several times

around the button with the help of a fine, sterilized needle. A cotton swab soaked with spore suspension was placed on the injured area. Fruits were then covered with perforated polythene bags and incubated at 25±1°C for seven days.

The cotton swab was moistened with sterile distilled water whenever it was deemed necessary. Typical symptoms of the rot were recorded from 3 to 7 days of inoculation.

2.2. Management strategy

The antagonistic potential of the fungal {*Trichoderma hamatum* (HP-20), *T. harzianum* (TG-1), *T. viride-1, T. viride-2, Gliocladium deliquescens, G. virens and Chaetomium globosum* (HP-29)}, yeasts {*Debaryomyces hansenii, Sporidiobolus pararoseus* (KFY-1)} and bacterial {*Bacillus subtilis, Pseudomonas fluorescens* (CHAO), *P. fluorescens* (Pf-1)} biocontrol agents were tested against *G. candidum* under *in vitro* conditions and those which found most effective were further explored to controlling the incited sour rot of Kinnow fruits.

2.3. In vitro evaluation of microbial antagonists against G. candidum

Evaluation of microbial antagonists against mycelial growth of the pathogen (*G. candidum*) was done by following standard methods (Sharma et al., 2012). The results were statistically analysed using a completely randomized design (CRD).

2.4. Fungal antagonists

2.4.1. Dual culture

Mycelial discs (5 mm diameter) cut from a seven-day-old actively growing culture of the pathogen and fungal antagonists were placed on potato dextrose agar (PDA) in the same Petri plate opposite to each other (approximately 6 cm apart). Plates having only the pathogen treated as a control. Each treatment has three replications. The inoculated plates were kept at 25 ±1°C in a BOD incubator. The radial growth of the pathogen from the disc towards the center of the plate was noted when the control plates were completely covered with mycelial growth of the pathogen. The % inhibition (PI) in mycelial growth of the pathogen by fungal antagonists over control was calculated.

2.4.2. Non-volatile metabolites

Prepared potato dextrose broth (PDB) medium filled in conical flasks was autoclaved, inoculated with each fungal antagonist, and kept in BOD incubator at 25±1°C. After 15 days of incubation, the broth was filtered through a three-layered 'Whatman' filter paper No. 42. Filtrate of each antagonist was mixed with PDA separately @ 10% and autoclaved in flasks. The PDA was then poured into sterile Petri plates aseptically. After the solidification of the PDA, the mycelial discs of 5 mm diameter were cut from the margin of a seven-day-old actively growing culture of the pathogen and inoculated in the center of the plates. Plates having only PDA without filtrate were treated as control. Each treatment has three replications. The inoculated plates were incubated at 25±1°C in a BOD incubator till the control plates showed full growth of the pathogen. The % inhibition (PI) in mycelial growth of the pathogen as compared to the control was calculated.

2.4.3. Volatile metabolites

Fungal antagonists and the pathogen were inoculated with 5 mm discs of seven days old actively growing culture in separate Petri plates on PDA. Lids of Petri plates were removed and the Petri plate containing the pathogen was inverted over the Petri plate containing the antagonist and sealed with adhesive tape (magic tape) under aseptic conditions.

The control has the same pathogen in both upper and bottom Petri plates. Inoculated Petri plates were incubated at 25±1°C in a BOD incubator. Each treatment has three replications. The % inhibition (PI) in mycelial growth of the pathogen as compared to the control was calculated.

2.5. Yeast and bacterial antagonists

Antifungal activity of yeasts and bacterial antagonists against the pathogen was tested by the "flooding plate technique" (Singh, 2004). The sterilized PDA medium was poured into Petri plates, and after solidification of the PDA, 0.5 ml of spore suspension (10^9 cfu ml^{-1}) of each antagonist was flooded separately in Petri plates, equally spread with a sterile bent (L-shaped) glass rod. After that, a mycelial disc of 5 mm diameter cut from the margin of a seven-day-old actively growing culture of the pathogen was inoculated in the centre of each plate. Plates having only PDA were treated as the control. Each treatment has three replications. The inoculated plates were kept in BOD incubator at 25±1°C. % inhibition (PI) in mycelial growth of the pathogen as compared to the control was calculated.

2.6. Biocontrol of sour rot

The fruits were surface sterilized and pricked through the 'pin prick method' up to a depth of 2 mm, making 5 wounds $fruit^{-1}$. These fruits were separately inoculated by dipping them in spore suspension (10^6 spores ml^{-1}) of the pathogen for 2 minutes. Spore suspension (10^9 cfu ml^{-1}) of each antagonist was used for dip treatment, both as pre- and post-inoculation treatments (Thompson, 1996; Kumari and Sharma, 2024). In the case of pre-inoculation treatment, the fruits were first dipped in the spore suspension of the antagonist for 5 minutes, air dried for 15 minutes and then inoculated with the pathogen, while in the post-inoculation treatment, the fruits were first inoculated with the pathogen and then treated with the antagonist. Fruits that were dipped in sterile distilled water at the place of the antagonistic spore suspension served as a control. The interval between inoculation and treatment with antagonists or vice-versa was 12 hr. Each treatment has three replications, containing seven fruits in each replication in a factorial randomized block design. The inoculated fruits were enclosed separately in pre-sterilized perforated polythene bags partially sealed with paper pins

and incubated at 25±1°C temperature and 90–100% RH. The number of wounds showing rotting was recorded on the 3rd and 6th day after inoculation (DAI). The percent rot incidence was calculated.

The percent rot reduction (PRR) in treated fruits compared to untreated control was calculated as described by Sharma et al. (2020).

3. Results and Discussion

3.1. Identification of the causal agent and incited fruit rot

Isolated fungus from the rotted portion of the fruits proved their pathogenicity and was identified by the ITCC (IARI), New Delhi, as *Geotrichum candidum* Link. (ITCC No. 6130). The symptoms of the incited fruit rot were similar to sour rot as described by Ashebre (2015).

3.2. Management

Microbial antagonists were tested against the sour rot pathogen (*Geotrichum candidum*) and potential antagonists were explored to manage the incited sour rot in kinnow fruits.

3.2.1. Effect of microbial antagonists on the mycelial growth of the G. candidum

3.2.1.1. Fungal antagonists

All antagonists significantly reduced the mycelial growth of the pathogen as compared to the control. Mean of all three techniques used revealed that, except for *C. globosum* all other antagonists reduced more than 50% growth of *G. candidum*. *Trichoderma hamatum* proved most effective, reducing the maximum growth (63.70%) of the *G. candidum*, followed by *T. viride*-1, which reduced 62.84% growth. *Gliocladium virens* and *T. harzianum* (TG-1) were found to be the next in order of decreasing efficacy, showing 60.80 and 59.94% reduction accordingly (Table 1, Plate 1a, b, and c).

Table 1: *In vitro* antagonism of different mycobial biocontrol agents against *G. candidum*

Biocontrol agents	% inhibition (PI)* in mycelial growth			
	Dual culture method	Volatile method	Non-volatile method	Mean
Trichoderma hamatum (HP-20)	69.26 (56.33)	63.52 (52.84)	58.33 (49.80)	63.70 (52.99)
T. harzianum (TG-1)	62.96 (52.51)	51.30 (45.75)	65.56 (54.07)	59.94 (50.78)
T. viride-1	68.52 (55.87)	61.85 (51.86)	58.15 (49.69)	62.84 (52.47)
T. viride-2	63.70 (52.95)	51.48 (45.85)	56.67 (48.83)	57.28 (49.21)
Gliocladium deliquescens	62.96 (52.51)	51.67 (45.96)	61.11 (51.42)	58.58 (49.96)
G. virens	70.93 (57.37)	53.70 (47.12)	57.78 (49.48)	60.80 (51.32)
Chaetomium globosum (HP-29)	48.52 (44.14)	12.04 (20.06)	34.26 (35.80)	31.61 (33.33)
Control	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)
SEm±	0.87	0.88	0.58	
CD (*p*=0.05)	2.62	2.64	1.73	
CV (%)	3.25	3.94	2.36	

* Average of three replications; Figures in parentheses are angular transformed values

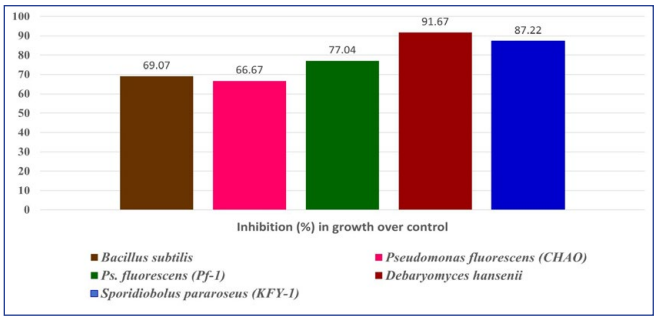

Figure 1: *In vitro* antagonism of microbial biocontrol agents against *G. candidum*

3.2.1.2. Yeast and bacterial antagonists

The yeast and bacterial antagonists significantly reduced the mycelial growth of *G. candidum* as compared to the control. Maximum growth reduction was shown by the yeast antagonists viz., *D. hansenii* and *S. pararoseus* (KFY-1), which exhibited 91.67 and 87.22% respectively, and differed non-significantly from each other (Figure 1, Plate 2). Among bacterial antagonists, the maximum growth reduction (77.04%) was rendered by bio-inoculant *P. fluorescens* (Pf- 1) followed by *B. subtilis,* i.e., 69.07%.

3.2.2. Effect of microbial antagonists on sour rot incidence in kinnow fruits

Fruits treated by dipping in the spore suspension of antagonists showed significantly lower incidence of fruit rot as compared to untreated control fruits. All antagonists were found to be more effective in controlling the fruit rot when applied as pre- inoculation than post-inoculation treatment. Incubation period was positively correlated with fruit rot incidence, where incidence on 6th DAI was significantly higher than on 3rd DAI (Table 2).

3.2.3. Pre-inoculation treatment

All the antagonists reduced the sour rot incidence as compared to untreated control; yeast antagonists proved more effective than bacterial and fungal antagonists. *Debaryomyces hansenii* reduced maximum (88.30%) sour rot incidence, followed by *S. pararoseus* (KFY-1), which showed 84.11% reduction in sour rot incidence over the untreated control. *Pseudomonas fluorescens* (Pf-1) and *B. subtilis* were found to be the next in decreasing order of bioefficacy against the fruit rotting, which retarded 79.94 and 77.15% sour rot incidence, respectively.

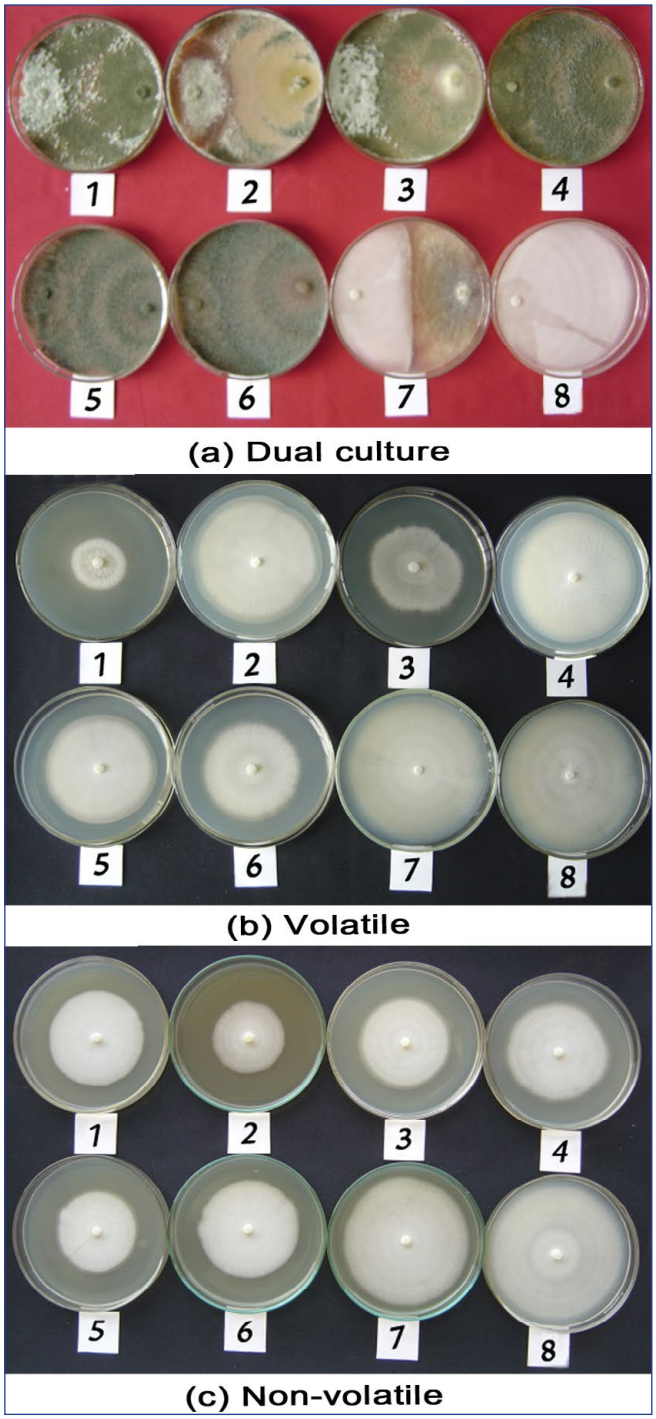

Plate 1: *In vitro* evaluation of fungal biocontrol agents against *G. candidum;* 1. *Trichoderma hamatum* (HP-20); 2: *T. harzianum* (TG-1); 3: *T. viride*-1; 4: *T. viride*-2; 5: *Gliocladium deliquescens*; 6: *G. virens*; 7: *Chaetomium globosum* (HP-29); 8: Control

The fungal antagonists, viz., *T. hamatum* and *T. viride*-1, also showed bioefficacy in controlling the fruit rotting by reducing 61.83 and 59.32% incidence of sour rot. At 3rd and 6th DAI, the sour rot incidence was 25.99 and 34.90%, respectively, which differed significantly.

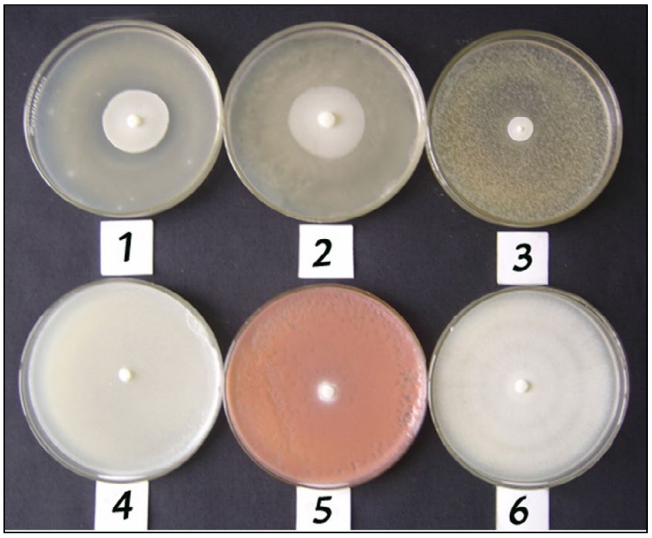

Plate 2: *In vitro* evaluation of bacterial and yeast biocontrol agents against G. candidum; 1. *Bacillus subtilis*; 2: *Pseudomonas fluorescens* (CHA); 3: *Ps. fluorescens* (Pf-1); 4: *Debaryomyces hansenii*; 5: *Sporidiobolus pararoseus* (KFY-1); 6: Control

3.2.4. Post-inoculation treatment

Though all antagonists tested reduced the sour rot incidence significantly as compared to the control. Maximum reduction (85.32%) in fruit rotting was observed in fruits which treated with the spore suspension of *D. hansenii,* followed by the treatment of *S. pararoseus* (KFY-1), which reduced 78.79% fruit rotting over the untreated control. Bacterial antagonists, viz., *P. fluorescens* (Pf-1) and *B. subtilis,* were found to be the next in decreasing order of bioefficacy, which retarded 74.18 and 69.84% sour rot incidence, respectively. The fungal antagonists, namely *T. viride*-1 and *T. hamatum,* showed low to moderate bioefficacy against fruit rotting. Sour rot incidence at 3rd DAI (29.39%) was significantly higher than at 6th DAI (42.66%).

Antagonistic microorganisms used as pre-inoculation treatment showed more efficacy than post-inoculation treatment. It might be due to having sufficient time to establish themselves in the host against the pathogen in case of pre-inoculation treatment. These results are in conformity with those of Sharma (2000) and Maharshi et al. (2009), who established the biocontrol potential of *D. hansenii* and *S. pararoseus* against citrus fruit rotting.

In addition to competition with the pathogen for nutrients and space, the antagonistic yeasts degrade the cell wall of the pathogen to some extent, mainly through glucanase activity and parasitization (Wisniewski et al., 1991).

Similar to the present findings, Spadaro et al. (2002) and Kaur et al. (2021) have also established the bioefficacy of *P. fluorescens* and *B. subtilis* against post-harvest fungal rotting. The biocontrol potential of these bioagents might be due to producing antibiotics like '2, 4-diacetyl-phloroglucinol' and 'Iturin' respectively, which have a wide antifungal spectrum (Reddy et al., 2009).

Table 2: Effect of different biocontrol agents on sour rot incidence in post-harvest treated kinnow fruits

Biocontrol agents	% Sour rot incidence in pre-inoculation treatment at (days)				% Sour rot incidence in post-inoculation treatment at (days)			
	3	6	Mean	DRI	3	6	Mean	DRI
Trichoderma hamatum (HP-20)	29.53 (32.90)	35.72 (36.70)	32.63 (34.80)	61.83	32.39 (34.67)	49.05 (44.45)	40.72 (39.56)	53.53
T. viride-1	31.91 (34.37)	37.62 (37.82)	34.77 (36.10)	59.32	35.24 (36.41)	51.43 (45.82)	43.34 (41.12)	50.54
Bacillus subtilis	15.72 (23.34)	23.34 (28.87)	19.53 (26.11)	77.15	20.00 (26.53)	32.86 (34.97)	26.43 (30.75)	69.84
Pseudomonas fluorescens (Pf-1)	13.34 (21.39)	20.96 (27.21)	17.15 (24.30)	79.94	17.62 (24.79)	27.62 (31.69)	22.62 (28.24)	74.18
Debaryomyces hansenii	8.09 (16.41)	11.91 (20.07)	10.00 (18.24)	88.30	10.48 (18.80)	15.24 (22.96)	12.86 (20.88)	85.32
Sporidiobolus pararoseus (KFY-1)	10.48 (18.85)	16.67 (24.06)	13.58 (21.46)	84.11	14.77 (22.59)	22.39 (27.80)	18.58 (25.20)	78.79
Control	72.86 (58.63)	98.10 (85.54)	85.48 (72.09)	0.00	75.24 (60.18)	100.00 (90.00)	87.62 (75.09)	0.00
Mean	25.99 (29.41)	34.90 (37.18)			29.39 (32.00)	42.66 (42.53)		
Source	SEm±	CD (p=0.05)	CV (%)		SEm±	CD (p=0.05)	CV (%)	
	0.56	1.63	4.12		0.68	1.97	4.45	
Days	0.30	0.87			0.36	1.05		
Biocontrol agents×Days	0.79	2.30			0.96	2.79		

Yeasts and bacterial antagonists were found to be more successful than fungal antagonists in Kinnow fruit rotting during the present study. Similarly, bioefficacy of yeast antagonists has been established by Rodrigues et al. (2024). According to Wisniewski and Wilson (1992), the bioefficacy of yeasts and bacterial antagonists might be due to their rapid growth, which is why they colonize the wound site where infection occurs and out-compete post-harvest pathogens for space and nutrients. Yeasts are drought-tolerant and therefore can survive in a dormant condition on the fruit surface under adverse environmental conditions, and yeasts produce extracellular materials (mostly polysaccharides) that not only enhance their survival but are also rendered antifungal activity (Droby et al., 1989 and Droby et al., 1996).

4. Conclusion

Dipping treatment of Kinnow fruits with spore suspension (10^9 cfu ml^{-1}) of antagonistic yeast like *D. hansenii* or local isolate of *S. pararoseus* (KFY-1) was recommended for biocontrol of the post-harvest sour rot caused by *G. candidum*. Since *D. hansenii* was appeared to control sour rot without antibiotic production, it was the best among the antagonists identified to gain acceptance commercially. The biocontrol of sour rot of kinnow fruits through these microbial antagonists may have commercial importance too, after large-scale testing with suitable formulations.

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