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# Effect of seed treatment on seed borne fungi of rice

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### Abstract

Farmers of our country are not aware about seed treatment for controlling seed borne fungi. Seeds of 3 rice varieties viz. BR11, BRRI dhan30 and BRRI dhan33 were collected from farmers houses of Gopalpur, Tangail. Three seed treating fungicides viz. vitavax 200 vitavax 200 (0.25%), thiovit (0.25%) and cupravit (0.25%) were used. Collected seed samples were tested by dry inspection and blotter method. The highest and the lowest germination were recorded in seed samples of BRRI dhan30 (90%) and BRRI dhan33 (75%). After seed treatment the highest and the lowest germination were recorded in seed samples of BRRIdhan30, BRRI dhan33 (100) treated by vitavax 200 (0.25%) and BR11 (80%) in control. Seed health test by blotter method revealed seed borne fungi belonging to six genera viz. Bipolarisorvzae (2.5 to 8.53%), Alternariapadwickii (5.3 to 13.35%), Fusariummoniliforme (11.66 to 21.67%), Fusariumoxysporum (1.25 to 4.35%), Curvularialunata (1.95 to 7.5%) and Aspergillusspp (1.75 to 6.54%) were encountered. Bur after seed treatment remarkable improvement viz. Bipolarisoryzae (0.15 to 3.75%), Alternariapadwickii (0.0 to 3.0%), Fusariummoniliforme (2.16 to 5.83%), Fusariumoxysporum (0.0 to 3.0%), Curvulariaunata (0.0 to 2.56%) and Aspergillusspp (0.0 to 1.5%) were encountered. Vitavax 200 was found most effective against the seed borne pathogens of rice. Nowadays, many botanical extracts such as neem, nishinda, garlic, alamonda and biological agent such as Trichoderma also using as seed treating agent and resulted significant higher germination and plant stand, less disease incidence and higher yield of different crops. So, the farmers should treat their seed before sowing in their field and through this technique they will be more benefited.

Key words: Dry inspection, blotter method, seed treatment, seed borne pathogen, vitavax 200.

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## Introduction

Rice (*Oryza sativa L.*) is the staple food of Bangladeshi people and it constituted about 85% of the total food grain production. It covers about 75% of the total cultivable land in Bangladesh (Ahmed *et al.*, 2013). In Aman season, rice covers about 55,30,253 ha land in Bangladesh and the production is 1,30,23,312 tons which is 2.35 MT/ha (BBS, 2014).The average world yield of rice is 3.84 tons/ha (Ahmed *et al.*, 2013). But the average yield of rice in Bangladesh is only 2.98 tons/ha. The demand for rice is constantly rising in Bangladesh with nearly 1.56% people being added each year (CIA World Fact book, 2011). Nazrul *et al.*,(2010) reported lack of healthy rice seed is considered as one of the most important constraints to rice production and productivity in Bangladesh. Most seed borne diseases like brown leaf spot, rice blast, stem rot and bacterial leaf blight are caused by the pathogens like Drechslera orvzae, Fusarium moniliforme, Pyricularia orvzae. Rhizoctonia solani, Sarocladium oryzae, Sclerotium oryzae, Trichoconiella padwickii and Xanthomonas compestris pv oryzae (Bhutta and Hussain 1998; Khan et al., 1990; Wahid et al., 2001, Gill et al., 1999) are the main causes of rice yield reduction in our country. In Bangladesh, approximately 2.5 million tons of rice worth more than Tk. 1200 millions lost annually due to diseases caused by seed borne pathogens (Fakir et al., 2003). (Bhuiyan et al., 2013) reported that a total of seven seed-borne fungi were associated with 40 rice (Oryza sativa) seed samples (cv. BR11 and BRRI dhan28) collected from two upazilas (Narshingdi Sadar and Shibpur) of Narshingdi district in Bangladesh. The identified fungal species were Bipolaris oryzae, Alternaria padwickii, Sarocladium orvzae, Curvularia lunata, Aspergillus niger and Fusarium spp. These pathogens are known to cause damage at different stages like storage, seed germination, seedling establishment, vegetative growth and reproductive phase. A total of 153 seed-borne pathogens were detected from rice of which 18% are of quarantine importance, 65% are native and 17% are storage pathogens (IRRI, 1987). The infected seeds may fail to germinate, transmit disease from seed to seedling and from seedling to growing plants (Fakir et al., 2002). Seed health is a well recognized factor in the modern agricultural science for desired plant population and good harvest. Seed treatment by chemicals is the best, environmentally safe and economical way to keep good seed health condition, because in this management practice mostly a very low dose (1-1.5 g/kg) of chemicals were used as compared to foliar application. Bhuiyan et al. (2013) detected seed treating fungicides viz. vitavax-200, bavistin 50 WP and captan were tested to control seed-borne fungi. Seed treatment with vitavax-200 @ 0.3% of seed weight eliminated all the seed-borne fungi and increased seed germination by 25.70% over control. Musyimi et al., (2012) conducted a study to evaluate the efficacy of fungicides, biological agents and host resistance in managing FHB and the associated T-2 toxin. Fungicides Pearl (500g/L carbendazim), Cotaf (50g/L hexaconacole), thiovit (micronized sulphur 80% w/w) and Folicur (430 g/L tebuconazole) were the standard checks. Fungicides resulted in 100% inhibition of pathogen radial growth in in vitro while microbial agents suppressed pathogen growth by up to 53%. Thiovit and Trichoderma were the most effective in reducing FHB (Fusarium Head Blight) severity in green house pot experiments. By keeping in view the above mentioned facts, the present study was carried out to determine the influence of different management practice on the prevalence of seed borne pathogens and to find out the most effective seed treating chemicals in reducing the pathogens.

### **Materials and Methods**

The experiment pertaining to the present investigation was carried out in the Seed Pathology Centre (SPC), Bangladesh Agricultural University (BAU), Mymensingh and MS Laboratory of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh during February -May 2009. 10 seed samples of each of viz. BR11, BRRI dhan30 and BRRI dhan33 were collected during T. Aman season from the farmer's houses of Gopalpur, Tangail. Seed samples were collected in polythene bags and after collection seed samples were placed in clean brown paper bag, labeled properly and preserved at 5-8°c temperature in refrigerator for subsequent use.

### Dry inspection of seed

For dry inspection from each sample 400 seeds were visually inspected. In a clean laboratory table, the seeds of each working sample were separated and 400 seeds were randomly separated first, and then graded them into five categories. The categories were a) Apparently healthy seeds, b) Discolored seeds, c) Spotted seeds, d) Unfilled seeds and e) Deformed seeds. The results were expressed in percentage.

### Seed health test by blotter method

To detect the seed borne pathogens Blotter method was used by following international rules for seed testing (ISTA, 1996). In this method, three layers of blotting paper (Whatman filter No. 1) were soaked in sterilized water and placed at the bottom of 9 cm diameter plastic and thereafter 25 seeds were placed per plate. 16 replicates (Petri dishes) were used for each sample where 400 seeds were placed. The seeds in the Petri dishes were incubated at  $20\pm2^{\circ}c$  under alternating cycles of 12 hours near ultraviolet (NUV) darkness for 7 days.

### Inspection of incubated seed samples

Each individual incubated seed was observed under stereomicroscope at 16x and 25x magnifications in order to record the incidence of seed borne fungi. Most of the associated seed borne fungi were detected by observing their growth characters on the incubated seeds on blotter paper following the keys outlines by Ramnath *et al.*, (1970). For proper identification of fungi temporary slides were prepared from the fungal colony and observed under compound microscope and identified with the help of keys suggested by Malone and Muskette (1964), Ellias (1971) and Neergaard (1979). The fungi from the incubated seeds were also transferred to PDA when needed. The culture was incubated at  $25\pm1^{\circ}$  cfor 3-7 days. Temporary slides were prepared from the fungal colony and observed under compound microscope to identify the fungi (Ellias, 1971; Agarwal *et al.*, 1989). The results were presented as percent incidence of individual pathogen.

# Preparation of fungicidal solutions and seed treatment with fungicides

Three different types of fungicides viz. vitavax 200 (0.25%), thiovit (0.25%) and cupravit (0.25%) were taken in three different conical flasks, then distilled water was poured in the conical flasks while it was shaken continuously and finally the volume of the solutions were made up to 100 ml. Thus 0.25% vitavax 200, thiovit and cupravit solutions were made and used for seed treatment. For seed treatment with fungicidal solutions the seeds were soaked in the solution for 1 hour. Khalequzzaman et al. (2008) reported the efficacy of two fungicides namely vitavax 200 and bavistin 50 WP, the best treatment was dipping seeds in 0.25% suspension of vitavax 200 for 3 hours, which was followed by dipping for 1 hour. After 1 hour the fungicidal solutions were drained out and the moistened seeds were kept in the blotter paper to remove excess moisture from the seed surface. Then the seeds were ready for plating in the blotter method. Treatments that are used for controlling seed borne fungi were as follows:

> T<sub>1</sub>: Vitavax 200 (0.25%), T<sub>2</sub>: Thiovit (0.25%) T<sub>3</sub>: Cupravit (0.25%)

#### Statistical analysis

The experiments were conducted following the Completely Randomized Design (CRD). The data collected from the experiment were analyzed for test of significance and compared the treatment means by using Least Significant Difference Test (LSD) as here less than 5 treatments were used.

### **Results and Discussions**

### Dry inspection

The results of dry inspection of collected seed samples of three rice varieties are presented in Table 1. Both the highest (83.35%) and the lowest (65.50%) apparently healthy seed were recorded in sample of BRRI dhan30. The highest (7.15%) and the lowest (2.16%) discolored seed was recorded in sample of BR11. The highest (18.93%) and the lowest (2.75%) spotted seed was recorded in sample of BRRI dhan30. The highest (8%) unfilled seed was recorded in BRRI dhan33 and the lowest (2.15%) was recorded in sample of BRRI dhan30. The highest (9.42%) deformed seed was recorded in BR11 and the lowest (1.18%) was recorded in sample of BRRI dhan33.Germination of the seeds varied from 75% to 90%. The highest germination (90%) was found in BRRI dhan30 and the lowest (75%) was in BRRI dhan33. Most of the seed samples had shown germination percentage between 75% to 85%.

# Prevalence of seed borne fungi obtained through blotter incubation tests

The incidence of seed borne fungal pathogens from the seeds collected from Gopalpur, Tangailare presented in Table 2. The fungal species belonging to six genera that were found in seed samples are Fusarium moniliforme, Bipolaris oryzae, Fusarium oxysporum, Curvularia lunata, Alternaria padwickii, and Aspergillus spp. The highest incidence of F. moniliforme (20.87%) was found in variety BRRI dhan33 and the lowest (11.66%) incidence was found in variety BR11. The highest incidence of B. oryzae (8.53%) was found in variety BRRI dhan33 and the lowest (2.50%) incidence was found in variety BR11. The highest incidence of F. oxysporum (4.35%) was found in variety BRRI dhan33 and the lowest (1.25%) incidence was found in variety BRRI dhan33. The highest incidence of C. lunata (7.50%) was found in variety BRRI dhan30 and the lowest (1.95%) incidence was found in variety BRRI dhan33. The highest incidence of A. padwickii (12.85%) was found in variety BRRI dhan30 and the lowest (5.30%) incidence was found in variety BRRI dhan30. The highest incidence of Aspergillus spp. (6.54%) was found in variety BRRI dhan33 and the

lowest (1.75%) incidence was found in variety BRRI dhan30.

### Effect of seed treatment

The effects of different treatments on % germination of collected seed samples of rice varieties are presented in Table-3. Germination ranged from 80% to 100%.The highest 100% germination was recorded in vitavax 200 treated seeds of BRRI dhan30 and BRRI dhan33 and the lowest 80% was found in control of BR11. But according to the gazette published by Seed Wing, Ministry of Agriculture, Government of the Peoples Republic of Bangladesh (2010) the minimum percent germination for seed standardize 96%.

 

 Table 1. Dry inspection of farmers stored BR11, BRRI dhan30, BRRI dhan33 seed collected from Gopalpur, Tangail

Sample no.		Percent grain under different category							
		Healthy seed	Discolored seed	Spotted seed	Unfilled seed	Deformed seed			
BR11	1	77.25	3.50	11.58	4.00	3.67			
	2	80.58	2.58	10.35	4.33	2.16			
	3	72.66	3.50	13.56	3.50	6.78			
	4	75.50	3.58	8.75	2.75	9.42			
	5	78.58	2.25	13.00	3.58	2.59			
	6	78.66	3.00	12.62	3.41	2.31			
	7	72.50	2.16	11.75	6.92	6.67			
	8	68.82	7.15	15.25	7.50	1.28			
	9	69.50	4.50	17.13	2.41	6.46			
	10	75.25	6.20	7.92	3.62	7.01			
С	V(%)	14.00	5.84	9.83	5.91	9.67			
	11	73.52	2.25	15.12	3.50	5.61			
	12	68.35	6.35	18.80	4.75	1.75			
BRRI dhan30	13	65.50	4.32	18.93	5.50	5.75			
	14	75.36	6.35	10.35	2.15	5.79			
	15	78.92	3.00	11.50	3.52	3.06			
	16	79.25	3.35	10.32	5.16	1.92			
BRI	17	81.35	2.75	9.65	4.50	1.75			
	18	77.85	2.82	12.70	5.32	1.31			
	19	74.55	4.50	11.00	5.00	4.95			
	20	83.35	6.00	2.75	3.58	4.32			
CV(%)		19.67	5.56	16.66	3.77	6.52			
	21	65.53	3.50	16.52	8.00	6.45			
	22	68.75	2.75	14.00	7.25	7.25			
BRRI dhan33	23	75.80	4.92	12.85	5.25	1.18			
	24	72.83	4.33	11.25	6.50	5.09			
	25	77.35	4.50	13.12	3.50	1.53			
RI (	26	69.35	4.92	16.52	3.50	5.71			
BRI	27	78.30	2.41	10.58	4.00	4.71			
	28	80.00	3.00	9.66	4.30	3.04			
	29	75.50	3.58	7.85	6.25	6.82			
	30	78.00	4.00	11.75	3.85	2.40			
С	V(%)	16.94	3.13	9.83	5.82	7.85			

\*Data represent the mean of four replications. CV means co-efficient of variation

		uc	% Prevalence of seed borne fungi						
Sample	no.	Grmination (%)	Bipolar isoryzae	Fusariumo xysporum	Fusariummon iliforme	Curvularial unata	Alternari apadwickii	Aspergillus sp.	Total (%)
	1	75.83	3.50	1.94	15.50	2.50	7.50	2.50	33.44
	2	78.39	5.65	2.33	17.00	6.50	12.35	2.66	46.49
	3	80.35	7.30	3.16	13.50	3.00	11.00	2.83	40.79
	4	82.53	4.00	2.50	11.66	2.33	8.35	3.75	32.59
BR11	5	85.65	5.65	3.00	15.00	4.50	6.35	4.50	39.00
BR	6	79.55	4.50	2.00	18.83	3.00	9.59	5.00	42.83
	7	80.53	6.70	3.50	13.56	2.16	12.25	4.66	42.83
	8	82.56	5.60	2.33	20.33	3.16	11.75	4.50	48.17
	9	78.65	6.50	2.50	14.75	4.16	8.30	4.16	40.37
	10	79.75	2.50	3.50	18.50	5.66	13.00	3.83	46.99
CV	/(%)	9.51	5.37	2.03	9.63	5.17	8.20	3.14	18.71
	11	76.63	3.00	2.00	16.00	3.25	8.50	1.75	34.50
	12	80.29	5.34	2.75	15.50	7.50	11.55	3.40	46.04
	13	78.45	6.30	3.28	13.25	2.50	12.25	4.00	41.58
BRRI dhan30	14	85.58	5.25	2.65	12.76	2.83	8.50	2.75	34.74
dha	15	82.75	6.75	3.50	14.00	5.40	5.30	3.50	38.45
RI	16	79.55	3.55	1.25	19.85	3.50	10.50	4.25	42.90
BR	17	78.50	7.00	4.25	15.50	3.25	11.35	5.66	47.01
	18	81.66	6.50	1.35	21.67	2.58	12.85	4.85	49.80
	19	78.00	5.60	3.50	14.55	3.25	9.50	5.86	42.26
	20	90.79	3.30	3.40	17.50	6.55	12.50	2.85	46.10
CV	/(%)	14.97	5.22	3.48	10.06	6.22	8.28	4.60	18.16
	21	78.50	7.67	2.15	17.00	4.00	9.89	2.00	42.71
	22	80.00	4.78	3.27	16.15	6.85	12.65	3.25	46.95
	23	75.25	6.38	4.20	14.28	1.95	13.35	4.50	44.66
n33	24	85.58	4.36	2.50	11.89	2.76	6.96	3.25	31.72
dhaı	25	82.75	5.45	4.00	13.00	4.50	7.30	4.25	38.50
BRRI dhan33	26	80.65	4.25	2.15	21.15	3.50	11.00	4.50	46.55
BR	27	81.50	5.15	3.85	14.75	3.76	12.35	6.54	46.40
	28	78.76	8.53	1.30	20.87	3.68	11.25	3.89	49.52
	29	82.65	6.50	4.35	15.00	4.15	10.26	4.89	45.15
	30	85.75	3.35	2.78	16.25	5.95	11.25	3.57	43.15
CV	/(%)	11.41	5.69	3.64	10.65	5.00	7.46	4.24	17.93

Table 2. Percent incidence of seed borne infection of BR11, BRRI dhan30, BRRI dhan33 (Blotter method)

\*Data represent the mean of four replications. CV means co-efficient of variation.

	lts	Germination (%)	% Prevalence of seed borne fungi						
Variety	Treatments		Bipolaris oryzae	Fusariumoxys porum	Fusariummon iliforme	Curvularial unata	Alternaria padwickii	Aspergillus sp.	
	Control	80.00d	6.00b	5.16a	20.00a	6.50a	7.00a	4.00	
-	Vitavax	99.00ab	0.15f	0.33e	4.50ef	0.00f	0.00e	0.00	
BR1	Cupravit	95.50ab	2.50d	1.50cd	2.16h	1.50e	0.00e	0.00	
	Thiovit	96.00ab	3.00cd	1.20d	5.30de	0.00f	1.00d	0.00	
30	Control	85.00c	7.80a	4.50a	16.00b	3.50c	6.50a	4.00	
han3	Vitavax	100.00a	1.38e	0.00e	2.25h	0.00f	1.30d	0.00	
BRRI dhan30	Cupravit	97.50ab	3.75c	3.00b	2.50gh	0.00f	1.50d	1.50	
BR	Thiovit	97.25ab	2.75d	1.50cd	5.83d	0.00f	1.30d	0.00	
33	Control	85.75c	6.50b	2.00cd	12.50c	5.00b	4.50b	3.00	
han3	Vitavax	100.00a	1.30e	0.00e	2.50gh	0.00f	1.70d	0.00	
BRRI dhan33	Cupravit	94.00b	1.55e	2.30bc	3.00gh	2.56d	3.00c	0.00	
BR	Thiovit	95.00ab	2.75d	1.85cd	3.50fg	0.00f	2.95c	0.00	
	LSD	4.77	0.89	0.93	1.14	0.72	0.94	1.36	

**Table 3.** Effect of fungicides on the prevalence major seed borne fungal pathogens of rice seeds (BR11, BRRIdhan30, BRRI dhan33) As treatments are less than 5 so here LSD is used.

Means followed by the same letter did not differ significantly at the 5% level by LSD

So the germination percentage should be increased. The effects of different treatments on % infection by different seed borne fungi after seed treatment of seed samples are presented in Table 3. The maximum infection of Fusarium moniliforme (20%), Bipolaris oryzae (7.8%), Fusarium oxysporum (5.16%), Curvularia lunata (6.5%), Alternaria padwickii (7.0%), and Aspergillus spp (4.0%) were found in control and infection of Fusarium oxysporum (0%), Curvularia lunata (0%), Alternaria padwickii (0%), and Aspergillus spp. (0%), were not found and minimum infection of Fusarium moniliforme (2.16%), Bipolaris oryzae (0.15%), were found when seeds treated with vitavax 200, thiovit and cupravit (Table 3). Seed borne disease is one of the factors for substantial damages of rice. Apparently healthy seeds found in seed samples ranged from 65.50% to 83.35%. The highest (83.35%) pure seed was recorded in BRRI dhan30. It has been recorded that the collected seed samples contained 2.16% to 7.15% discolored, 2.75% to 18.93% spotted, 2.15% to 8.00% unfilled and 1.18% to 9.42% deformed. Fakir et al. (2002) recorded 91.20% to 98.89% pure

seed, 0.06% to 2.73% unfilled, 14.43% to 24.44% discolored seed, 33.72% to 37.71% spotted seed and 8.46% to 15.50% deformed seeds of rice collected from Rajshahi, Rangpur and Bogra in Bangladesh. Islam *et al.* (2007) reported that maximum pure seed (99.01%) was found in seed samples of trained farmers and minimum (96.19%) in untrained farmers.

In the present investigation 3 different treatments including a control were tested in blotter method, where their effect on germination and seed borne fungi were evaluated. All the treatments showed superior influence on germination of seeds over control (Table 2 & 3). Percent seed germination was increased in treated seeds compared to untreated seeds. The seed treated with the vitavax 200 shown highest percentage of germination and the other two fungicides also found to increase the germinations over control. They were statistically similar with vitavax 200. This is also in agreement with the findings of Islam (2000), Rahman (2000), Parisi (2001), Rashid (2007), Ashok Gaur (2005), Kabir (2006), Islam (2007). They found that the fungicide vitavax 200 shown excellent increase of germination. The percentage ranges of the seed borne fungi that were found in the seed samples after seed treatment were Bipolaris oryzae (0.15% to 3.75%), Fusarium oxysporum(0% to 3%), Fusarium moniliforme (2.16% to 5.83%), Curvulari alunata (0% to 2.56%), Alternaria padwickii (0% to 3%) and Aspergillus spp. (0% to 1.5%). The association of seed borne fungi of rice have also been reported by a good number of researchers (Mendoza and Molina, 1980; Mia and Mathur, 1983; Agarwal et al. 1990; Sisterna et al. 1994; Purushattam et al. 1996; Sharma et al. 1997; Bicca et al. 1998; Fakir, 2000; Naeem Khalid et al. 2001; Fakir et al. 2002, Rahman et al. 2002a, Rahman et al. 2002b, Fakir et al. 2003, Nahar 2003, Mathur et al. (2004), Portapuglia (2004) and Islam (2007). It was recorded from the study that all the fungicides appeared to be effective to control the growth of Curvularia lunata. The most effective result was found in inhibiting seed borne fungi Aspergillus sp. prevalence. All of the three fungicides could completely control the prevalence of seed borne fungi Aspergillus sp. Among the fungicides tested vitavax 200 was found the best and could completely controlled the prevalence of Fusarium oxysporum which is an observations made by Akanda and Fakir, 1985b. Vitavax 200 was found to be most effective for controlling Bipolaris oryzae, the most effective result was found in variety BR11 (0.15%). In killing Fusarium moniliforme cupravit was found to be most effective and the prevalence of the pathogen was ranged from 2.16% to 3.0%, the most effective result was found in variety BR11 (2.16%). Alternaria padwickii was found to be completely controlled when the seeds were treated with vitavax-200 (0.00% to 1.70%) and cupravit (0.00% to 3.00%). The most effective result was found in the variety BR11 (0%).Among the fungicides for controlling major seed borne fungi of rice, the fungicides vitavax 200 found to be most effective against the prevalence of seed borne pathogen. This is also in agreement with the findings of Islam (2000), Rahman (2000), Parisi (2001), Rashid (2007), Ashok Gaur (2005), Kabir (2006), Islam (2007). Jambhulkar et al. (2007) also found a lower performance of vitavax than others. Dayan (2005) revealed for cupravit, Rahman (2000) and Akter (2001) shown that thiovit gave satisfactory result over other fungicides.

The findings of the present studies revealed that vitavax 200 is the best for germination and also for controlling seed borne fungal pathogens but considering all other aspects performance of cupravit and thiovit were also satisfactory.

### Conclusion

Based on the findings of the present study, it may be concluded that health and quality of rice seeds collected from farmer's houses were not good. So seeds should be treated before use. The result of the present investigation revealed that seed treatment with different chemicals will be helpful to prevent germination failure. Among different seed treating agents vitavax 200 has been proven as the best seed treating agent in controlling different seed borne fungi which is considered as effective disease management strategy.

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