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In Vitro Inhibition of the Mycelial Growth of Some Root Rot Fungi by *Rhizobium leguminosarum* Biovar *phaseoli* Isolates

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Abstract: In solid medium, the effects of twenty-three *Rhizobium leguminosarum* biovar phaseoli isolates on the mycelium development of the phytopathogenic fungi of three different genera (*Fusarium oxysporum, Pythium ultimum* and *Rhizoctonia solani*) were tested in vitro. The experiments were achieved by applying the superimpose dual culture technique. Most of the rhizobia tested inhibited the fungal development. The inhibition rates displayed differences in accordance with the bacteria and fungi isolates utilized. However, the inhibitory effect of the rhizobia occurred most on the isolates of *Fusarium* and *Pythium* species (up to 30.35% and 29.75% respectively) and least on those of *Rhizoctonia* species (16.03%). The most effective *Rhizobium* isolate on the three fungal species was *Rhizobium leguminosarum* Çar 9.3.2 isolate and percent inhibitions were 14.65-16.03 for *R. solani* isolates and 14.62-30.35 for *P. ultimum* isolates and also 14.58-29.75 for *F. oxsporum* isolates.

Key Words: inhibition, *R. solani, P. ultimum, Fusarium oxsporum, Rhizobium leguminosarum*, root rot fungi

Bazı Kök Çürükçül Funguslarının Misel Gelişiminin *Rhizobium leguminosarum* Biovar *phaseoli* İzolatlarıyla in vitro İnhibisyonu

Özet: Katı ortamda 3 farklı cinse ait (*Fusarium oxysporum, Pythium ultimum* ve *Rhizoctonia solani*) bitki patojeni fungusun misel gelişimine yirmiüç *Rhizobium leguminosarum* biovar phaseoli izolatının etkisi in vitro da test edilmiştir. Denemeler Süper İmpose İkili Kültür Tekniği kullanılarak gerçekleştirilmiştir. Test edilen Rhizobiumların çoğu fungusların gelişimini inhibe etmiştir. İnhibisyon oranları kullanılan bakteri ve fungus izolatına göre değişme göstermiştir. Ancak kullanılan rhizobiumların inhibisyon etkisi en fazla *Fusarium, Pythium* (sırasıyla % 30.35 ve % 29.75) ve en az olarak da *Rhizoctonia* sp. (% 16.3) ait izolatları üzerine gerçekleşmiştir. Her üç fungal tür üzerine en etkili *Rhizobium* izolatı *Rhizobium leguminosarum* Çar 9.3.2 izolatı olup bu izolatın sırasıyla R. solani izolatları üzerine inhibisyon etkisi % 14.65-16.03, *P. ultimum* izolatları üzerine % 14.62-30.35 ve *F. oxysporum* izolatları üzerine % 14.58-29.75 olmuştur.

Anahtar Sözcükler: inhibisyon, *R. solani, P. ultimum, Fusarium oxsporum, Rhizobium leguminosarum*, kök çürükçül fungusları

Introduction

The bean, which is a widely consumed food in Turkey, is a large-seeded, yearly leguminous plant. This legume is grown in almost every part of Turkey. However, diseases limit bean production. In Samsun, one of the principal agricultural areas in Turkey, where beans are widely grown, one of the basic disease problems is root rot, which is caused by fungi of *Rhizoctonia*, *Fusarium* and *Pythium* species (1-2). In order to decrease the effects of these pathogens, various fungucides are widely used. As the chemical agents used to decrease the effects of diseases cause serious environmental pollution, in recent years biological control agents instead of chemical control agents are suggested (3-5). Nevertheless, for biological control agents to control root diseases, they must colonize the rhizosphere first (6).

Rhizobia are known as the bacteria colonizing the rhizosphere (7). The rhizosphere area in bean plants is mostly colonized by *Rhizobium leguminosarum*. These rhizobia display specific interactions with various soil pathogens, and the disease reactions caused by them display variations (8-10).

In a study on the use of *Rhizobium* species for this purpose, Buonassisi et al. (11) determined that the *Rhizobium* isolates they had isolated from beans inhibited especially *Fusarium* species, and they were not very effective on *Rhizoctonia* or *Pythium* species. Chao (10) demonstrated that *Rhizobium leguminosarum* biovar *phaseoli* was variably effective on the inhibition of the *Pythium*, *Fusarium* and *Rhizoctonia* species. Lalande et al. (12) explained that the inhibitory effect of *R. Leguminosarum* biovar viceae varied in accordance with the isolates used. Hassan Dar et al. (13) stated that *Rhizobium leguminosarum* isolates decreased the disease effects caused by *Fusarium*. Omar and Abd-Alla (14) stated that Rhizobia considerably inhibited the mycelial growth of *Fusarium solani* and *Rhizoctonia solani.*

The purpose in this study was to determine the in vitro inhibitory effect of 23 *Rhizobium* isolates obtained from bean plants collected from the Çarşamba district in Samsun (Turkey) on two different isolates of each species of *Rhizoctonia solani*, *Fusarium oxysporum* and *Pythium ultimum*, which cause various diseases in seedling plants of the bean.

Materials and Methods

Materials

Isolates of *Rhizobium leguminosarum* species, which cause nodules in Leguminosae, were used. *Phaseolus vulgaris* L. samples were collected from random parts of cultured bean fields in the Çarşamba district in Samsun. *R. Leguminosarum* isolates were obtained from the nodules in bean roots (15) and were established to be *Rhizobium leguminosarum* in accordance with the nodulation and other test results (16-19). The isolated bacterial isolates are shown in Table 1.

Table 1. The *Rhizobium* isolates obtained from fields

Order No	Isolate Code	Location of isolation
1	Çar. 1.1.	Village Bölmeçayır – Çarşamba – Samsun
2	Çar. 1.2.	Village Bölmeçayır – Çarşamba – Samsun
3	Çar. 2.1.2	Village Araplı - Çarşamba – Samsun
4	Çar. 2.3.1	Village Araplı - Çarşamba – Samsun
5	Çar. 2.3.3	Village Araplı - Çarşamba – Samsun
6	Çar. 3.1.1	Village Damlataş - Çarşamba – Samsun
7	Çar. 3.3.1	Village Damlataş - Çarşamba – Samsun
8	Çar.3.3.3	Village Damlataş - Çarşamba – Samsun
9	Çar. 4.1.1	Village Beylerce - Çarşamba – Samsun
10	Çar. 4.1.2	Village Beylerce - Çarşamba – Samsun
11	Çar. 5.1.1	Village Yeni Karacalı - Çarşamba – Samsun
12	Çar. 5.2.1	Village Yeni Karacalı - Çarşamba – Samsun
13	Çar. 5.2.2	Village Yeni Karacalı - Çarşamba – Samsun
14	Çar. 5.2.3	Village Yeni Karacalı - Çarşamba – Samsun
15	Çar. 5.3.2	Village Yeni Karacalı - Çarşamba – Samsun
16	Çar. 7.1.1	Village Kirazbucağı - Çarşamba – Samsun
17	Çar. 8.1.3	Village Yeni Köseli - Çarşamba – Samsun
18	Çar. 8.2.2	Village Yeni Köseli - Çarşamba – Samsun
19	Çar. 9.2.1	Yukarı Dikencik - Çarşamba – Samsun
20	Çar. 9.3.1	Yukarı Dikencik - Çarşamba – Samsun
21	Çar. 9.3.2	Yukarı Dikencik - Çarşamba – Samsun
22	Çar. 10.2.1	Village Karamustafalı - Çarşamba – Samsun
23	Çar. 10.3.2	Village Karamustafalı - Çarşamba – Samsun

In this study were used the pathogenic fungi that were isolated from the cultured fields in the Çarşamba district in a previous study (Table 2). During identification studies, *R. solani* isolates were inoculated onto PDA plates and incubated at 25 ± 1°C for 24-36 h. Then it was determined whether or not the developing colonies showed mycelial characteristics of *Rhizoctonia* species such as branching at right angles, constriction of the hypha at point of origin or union with main hyphae and presence of dolipore septa. Later, fresh hyphae were stained by Safranin O and the number of nuclei in a hyphal cell and the hyphal diameters were determined (20). *Fusarium* isolates were identified by taking into consideration the culture color in Potato sucrose agar (PSA) and the morphological properties in Synthetischer nahrstoffarmer agar (SNA) according to Booth (21). *Pythium* isolates were identified by taking

Table 2. The pathogenic fungus species isolated from the cultured bean fields

Fungal isolate code	Species of bean isolated from	Species of fungus
Çaf36B23	*Phaseolus vulgaris* L.	*Rhizoctonia solani* Kühn
Çaf46B31	*Phaseolus vulgaris* L.	*Rhizoctonia solani* Kühn
Çaf48B11	*Phaseolus vulgaris* L.	*Fusarium oxysporum* Schlechtend.:Fr f.sp. *phaseoli* J.B. Kendrick & W.C. Snyder
Çaf57B13	*Phaseolus vulgaris* L.	*Fusarium oxysporum* Schlechtend.:Fr f.sp. *phaseoli* J.B. Kendrick & W.C. Snyder
Çaf40B31	*Phaseolus vulgaris* L.	*Pythium ultimum* Trow
Çaf59B31	*Phaseolus vulgaris* L.	*Pythium ultimum* Trow

into consideration the properties of the sexual and asexual reproduction organs in water culture according to Dick (22). Pathogenicity test of the isolates were carried out using the seeds of *P. vulgaris* L. according to Ichielevitch-Auster et al. (23).

The Dual Culture Method (the in vitro inhibition test)

In our study, "the superimpose dual culture method" of Lalande and Bissonette (12) was used to determine the inhibitory effects of *Rhizobium* isolates on fungal development. The *Rhizobium* isolates activated in the YMA medium were inoculated into test tubes containing 5ml YMB (KH_2PO_4 0.5 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 0.1 g, mannitol 10 g, yeast extract 1 g, distilled water 1000 ml pH 6.8-7) and were left for incubation at 30°C for three days. The *Rhizobium* suspension (2.5ml/l) that developed following the three days' incubation was mixed into the YMA medium and the medium was poured into sterilized petri dishes. The medium containing *Rhizobium* that developed following the three days incubation at 30°C was covered with 10ml water agar (15g/l) after cooling by 45-50°C and then 7 mm discs, which were taken from the sides of the two-day-old fungus cultures, were placed on the solidified agar. The cultures without *Rhizobium* and inoculated only with fungi comprised the control group. The cultures were incubated at 30°C for five days and the fungal development and inhibition were recorded every day. The experiments were repeated five times. The results were subjected to variance analysis and Duncan's multi-range test.

Results

Identification of Isolates

The *Rhizoctonia* isolates showed the diagnostic mycelial characteristics for *Rhizoctonia*. The isolates were multinucleate and their hyphal diameters > 7 µm. Vegetative hyphae of the

isolates were brown, sclerotia were irregular in shape, light to dark brown, not differentiated into rind and medulla.

Colonies on PSA of *Fusarium* isolates covering the plate in 2 weeks had white felty mycelia with purplish areas, no exaduate, reverse colorless with purpish streaks radiating from the center. Hyphae were septate, hyaline. Conidiophores were sparse, not distinct, hyaline. Conidiogenous cells were phialidic, simple or on sparsely branched conidiopheres. Conidia were of two kinds: macroconidia were falcate, hyaline, generally 3 septate, basal cell not distinct; microconidia ovoid.

Colonies on potato carrot agar (PCA) of *Pythium* isolates showed a radiate pattern and formed cottony aerial mycelium on corn meal agar (CMA). Sporangia were not produced in water culture. Oogonia were globose, smooth walled, mostly terminal. Antheridia were mostly one per oogonium, terminal, sessile, closely monoclinous (originating from just below the oogonium). Oospores were single, globose, aplerotic and thick walled. In the pathogenity tests, the fungal isolates were highly pathogenic on *P. vulgaris* and caused severe hypocotyls and root rot.

Inhibition on Fungal Isolates

Data were determined in cm and the values were subjected to analysis of variance. According to the results, the inhibitory effects of various rhizobial isolates on the development of test fungi were found to be significantly variable (P=0.01). Although the fungal inhibition differed in accordance with the isolate used, in general it was observed that the *Fusarium* and *Pythium* isolates were more inhibited than the *Rhizoctonia* isolates (Table 3). Among the Rhizobial isolates, the inhibitory effect of the *R. Leguminosarum* Çar. 9.3.2 isolate on both isolates of *R. solani, P. ultimum* and *F. oxysporum* was high. Isolate *R. Leguminosarum* Çar. 9.3.2 reduced the mycelial growth of *R. solani* by 14.65 and 16.03, *P. ultimum* by 14.62 and 30.35, and *F. oxysporum* by 14.58, 29.75 respectively (Table 4). However, it was determined that the inhibitory effects of this isolate on *F. oxysporum* and *P. ultimum* isolates were higher. While hardly any rhizobial isolates had insignificant inhibitory effects on the *F. oxysporum* isolates, the inhibitory effects of the Çar. 5.1.1 (5.81% and 4.51%) and Çar. 8.2.2 isolates (2.09% and 6.32%) on *R. solani* and of Çar. 4.1.1 isolate (1.89% and 0.23%) on *P. ultimum* were found to be insignificant.

Discussion

In the results obtained in this study, which depended on inoculating the rhizobial isolates together with the pathogenic fungi, it appeared that the rhizobial isolates inhibited the development of the fungal pathogens and the inhibition rates varied according to the isolates used. It was pointed out by Chao (10) that the *Rhizobium leguminosarum* biovar *phaseoli* had an effect on the inhibition of the *Pythium*, *Fusarium* and *Rhizoctonia* species at various rates,

Table 3.1. The inhibition effects of the Rhizobial isolates on *R. solani* isolates

Bacteria (*R. leguminosarum*)		*R. solani*		
		Caf36B23(cm)	Caf46B31(cm)	Averages(cm)
Control		8.60 bc	8.86 ab	8.73a
1	Çar.1.1	8.04 cdefghij	8.30 bcdefgh	8.17 defg
2	Çar.1.2	8.02 cdefghijk	7.66 ijklm	7.84 gh
3	Çar.2.1.2	8.02 cdefghijk	7.86 fghijkl	7.94 efgh
4	Çar.2.3.1	8.02 cdefghijk	8.32 bcdefgh	8.17 defg
5	Çar.2.3.3	8.14 cdefghi	8.42 bcdefg	8.28 bcdef
6	Çar.3.1.1	7.72 hijklm	8.30 bcdefgh	8.01 defgh
7	Çar.3.3.1	7.98 defghijk	7.78 hijklm	7.88 fgh
8	Çar.3.3.3	8.04 cdefghij	9.28 a	8.66 ab
9	Çar.4.1.1	8.48 bcde	8.24 cdefghi	8.36 abcd
10	Çar.4.1.2	8.06 cdefghi	8.54 bcd	8.30 bcde
11	Çar.5.1.1	8.30 bcdefgh	8.46 bcdef	8.38 abcd
12	Çar.5.2.1	8.06 cdefghi	7.92 efghijk	7.99 defgh
13	Çar.5.2.2	8.10 cdefghi	8.14 cdefghi	8.12 defg
14	Çar.5.2.3	8.12 cdefghi	9.16 a	8.64 abc
15	Çar.5.3.2	8.28 cdefgh	8.24 cdefghi	8.26 cdef
16	Çar.7.1.1	7.94 defghijm	9.24 a	8.59 abc
17	Çar.8.1.3	7.28 m	9.24 a	8.26 bcdef
18	Çar.8.2.2	8.42 bcdefg	8.30 bcdefgh	8.36 abcd
19	Çar.9.2.1	8.10 cdefghi	7.46 jklm	7.78 gh
20	Çar.9.3.1	8.24 cdefghi	8.02 cdefghijk	8.13 defg
21	Çar.9.3.2	7.34 lm	7.44 klm	7.39 i
22	Çar.10.2.1	7.84 ghijklm	8.06 cdefghi	7.95 efgh
23	Çar.10.3.2	7.46 jklm	7.96 defghijk	7.71 hi
averages		8.25 B	8.30 A	
LSD= 0.01		Bacteria= 0.3472; fungi x bacteria interactions= 0.4910		

Table 3.2. The inhibition effects of the Rhizobial isolates on *P. ultimum* isolates.

Bacteria (*R. leguminosarum*)		*P. ultimum*		
		Caf59B31(cm)	Caf40B31(cm)	Averages(cm)
Control		8.48 a	8.50 a	8.49 a
1	Çar.1.1	7.26 ijklmn	8.42 ab	7.84 cd
2	Çar.1.2	7.38 fghijklmn	7.36 fghijklmn	7.37 defg
3	Çar.2.1.2	7.10 klmno	7.22 jklmn	7.16 fgh
4	Çar.2.3.1	7.00 mnop	7.76 bcdefghijkl	7.38 defg
5	Çar.2.3.3	7.88 abcdefghij	8.16 abcd	8.02 bc
6	Çar.3.1.1	7.12 klmno	8.02 abcdefg	7.57 def
7	Çar.3.3.1	8.00 abcdefgh	7.24 ijklmn	7.62 cdef
8	Çar.3.3.3	6.52 op	7.02 mnop	6.77 hi
9	Çar.4.1.1	8.32 abc	8.48 a	8.40 ab
10	Çar.4.1.2	7.92 abcdefghi	7.58 defghijklm	7.75 cde
11	Çar.5.1.1	7.42 efghijklmn	7.24 ijklmn	7.33 efg
12	Çar.5.2.1	7.08 lmno	7.68 cdefghijklm	7.38 defg
13	Çar.5.2.2	6.80 nop	7.34 ghijklmn	7.07 gh
14	Çar.5.2.3	8.08 abcde	7.04 abcdef	8.06 bc
15	Çar.5.3.2	7.08 lmno	7.68 cdefghijklm	7.38 defg
16	Çar.7.1.1	7.56 defghijklm	7.34 ghijklmn	7.45 defg
17	Çar.8.1.3	7.56 defghijklm	7.34 ghijklmn	7.45 defg
18	Çar.8.2.2	7.12 klmno	8.02 abcdefg	7.57 def
19	Çar.9.2.1	7.42 efghijklmn	7.14 klmno	7.28 efg
20	Çar.9.3.1	7.80 bcdefghijk	7.28 ijklmn	7.54 defg
21	Çar.9.3.2	7.24 ijklmn	5.92 q	6.58 i
22	Çar.10.2.1	7.32 hijklm	7.68 cdefghijklmn	7.50 defg
23	Çar.10.3.2	6.42 pq	6.74 nop	6.58 i
Averages		7.412 B	7.55 A	
LSD= 0.01		Bacteria= 0.3472; fungi x bacteria interactions= 0.4910		

Table 3.3. The inhibition effects of the Rhizobial isolates on *F. oxysporum* isolates

Bacteria (*R. leguminosarum*)		*F. oxysporum*		
		Caf48B11(cm)	Caf57B13(cm)	Averages(cm)
Control		8.70 a	8.58 ab	8.64 a
1	Çar.1.1	8.70 a	6.46 lmn	7.58 bcde
2	Çar.1.2	8.22 abcd	7.48 defghi	7.85 bc
3	Çar.2.1.2	7.48 defghi	7.20 ghijk	7.34 def
4	Çar.2.3.1	7.40 efghij	8.22 abcd	7.81 bcd
5	Çar.2.3.3	7.76 cdefgh	7.98 abcdef	7.87 bc
6	Çar.3.1.1	7.16 hijkl	6.98 ijklm	7.07 fg
7	Çar.3.3.1	8.12 abcde	8.00 abcdef	8.06 b
8	Çar.3.3.3	6.44 mn	6.22 n	6.33 h
9	Çar.4.1.1	8.04 abcdef	7.92 bcdefg	7.98 b
10	Çar.4.1.2	6.50 klmn	7.58 defghi	7.04 fg
11	Çar.5.1.1	8.00 abcdef	6.56 klmn	7.28 ef
12	Çar.5.2.1	8.12 abcde	7.46 efghi	7.79 bcd
13	Çar.5.2.2	7.04 hijklmn	7.54 defghi	7.29 ef
14	Çar.5.2.3	7.96 bcdef	7.74 cdefgh	7.85 bc
15	Çar.5.3.2	8.12 abcde	7.46 efghi	7.79 bcd
16	Çar.7.1.1	7.68 defghi	7.52 defghi	7.60 bcde
17	Çar.8.1.3	7.68 defghi	7.52 defghi	7.60 bcde
18	Çar.8.2.2	7.16 hijkl	6.98 ijklm	7.07 fg
19	Çar.9.2.1	7.38 efghij	7.50 defghi	7.44 cdef
20	Çar.9.3.1	8.46 abc	7.94 bcdefg	8.00 b
21	Çar.9.3.2	7.38 efghij	6.02 n	6.70 gh
22	Çar.10.2.1	8.46 abc	7.60 defghi	8.03 b
23	Çar.10.3.2	6.70 jklmn	7.34 fghij	7.02 fg
Averages		7.678 A	7.408 B	
LSD= 0.01		Bacteria= 0.3472; fungi x bacteria interactions= 0.4910		

Table 4. Percent inhibition rates of Rhizobium izolates on 3 diffferent fungi species

Bacteria (*Rhizobium*)		*R. solani*		*P. ultimum*		*F. oxysporum*	
		Caf36B23	Caf46B31	Caf59B31	Caf40B31	Caf48B11	Caf57B13
1	Çar.1.1	6.51	6.32	14.39	0.94	0.00	24.62
2	Çar.1.2	6.74	13.54	12.97	13.41	4.86	12.72
3	Çar.2.1.2	6.74	11.29	16.27	15.06	13.42	15.98
4	Çar.2.3.1	6.74	6.09	17.45	8.70	14.35	4.08
5	Çar.2.3.3	5.35	4.97	7.07	4.00	10.18	6.88
6	Çar.3.1.1	10.23	6.32	16.04	5.65	17.13	18.55
7	Çar.3.3.1	7.21	12.19	5.66	14.82	6.02	6.65
8	Çar.3.3.3	6.51	0.00	23.11	17.41	25.46	27.42
9	Çar.4.1.1	1.39	7.00	1.89	0.23	6.94	7.58
10	Çar.4.1.2	6.28	3.61	6.60	13.18	24.77	11.55
11	Çar.5.1.1	5.81	4.51	12.50	14.82	7.41	23.45
12	Çar.5.2.1	6.28	10.61	16.50	9.65	6.02	12.95
13	Çar.5.2.2	5.81	7.22	19.81	13.65	18.52	12.02
14	Çar.5.2.3	5.58	0.00	4.72	5.41	7.87	9.68
15	Çar.5.3.2	3.72	7.00	16.50	9.65	6.02	12.95
16	Çar.7.1.1	12.32	0.00	10.85	13.65	11.11	12.25
17	Çar.8.1.3	15.35	0.00	10.85	13.65	11.11	12.25
18	Çar.8.2.2	2.09	6.32	16.04	5.65	17.13	18.55
19	Çar.9.2.1	5.81	15.80	12.50	16.00	14.58	12.48
20	Çar.9.3.1	4.19	9.48	8.02	14.35	7.41	7.12
21	Çar.9.3.2	14.65	16.03	14.62	30.35	14.58	29.75
22	Çar.10.2.1	8.84	9.03	13.68	9.65	2.08	11.32
23	Çar.10.3.2	13.25	10.16	24.29	20.70	22.45	14.35

Percent inhibition = [(A-B)/A]x100
A= Control measurement B = averages of repetition numbers

and by Lalande et al. (12) that the inhibitory effect of the *R. leguminosarum* biovar viceae in accordance with the isolates used. Also in a recent study, Omar and Abd-Alla (14) determined that rhizobia significantly inhibited the mycelial growth of *Fusarium solani* and *Rhizoctonia solani*. In addition, it was observed in our study that the inhibitory effect of the rhizobial isolates were generally more effective on the *Fusarium* and *Pythium* species and less effective on the *Rhizoctonia* isolates. These results seem to be parallel to those of the study by Buonassisi et al.

(11) and those of the study by Özkoç and Hatat (24), which was a pre-study on a few *Rhizobium* and *Bradyrhizobium* isolates of various legume plants.

In consequence, with our study and in the light of the findings by the above- mentioned researchers, we may reach the following conclusion: it is observed that rhizobial bacteria might protect the plant with which they make symbiotic contact against the root rot pathogenic fungi, especially *Pythium*, *Fusarium* and *Rhizoctonia*, by suppressing their pathogenic effects, even in certain limits. The inhibitory effects of the rhizobial isolates vary according to the strain. Due to that variation, in future it will be easier to form appropriate organism combinations in biological control by the determination of appropriate biotypes.

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