



P-ISSN: 2349-8528

E-ISSN: 2321-4902

www.chemijournal.com

IJCS 2020; 8(4): 1481-1485

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Received: 10-05-2020

Accepted: 12-06-2020

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Evaluation of different fungicides against foliar blight complex of onion *in vitro*

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DOI: <https://doi.org/10.22271/chemi.2020.v8.i4n.9821>

Abstract

Onion (*Allium cepa* L.) is one of the most widely used vegetable due to its flavouring and seasoning the underground vegetable, both at mature and immature bulb stage in tropical and sub tropical countries. The foliar blight complex of onion resulted due to combined infection of *Alternaria porri* and *Stemphylium vesicarium*. Narrow spectrum fungicides viz., tebuconazole 25.9 EC and propiconazole 25 EC proved superior (84.07 and 81.94 % growth inhibition, respectively) against the combined radial growth of *A. porri* and *S. vesicarium* under laboratory condition. Rests of the fungicides were also found effective to inhibit the growth of the fungus. Among broad spectrum non-systemic fungicides, propineb 70 WP resulted in 79.07 % growth inhibition and among compound fungicides, highest growth inhibition was recorded in tebuconazole 50 WG + trifloxystrobin 75 WG followed by carboxin 37.5 DS + thiram 37.5 DS, carbendazim 12 WP + mancozeb 63 WP.

Keywords: Onion, *Alternaria porri*, *Stemphylium vesicarium*

1. Introduction

Onion (*Allium cepa* L.) rightly called as “queen of kitchen” is one of the oldest and an important spice, cool season and sensitive to photoperiod crop grown in India as well as tropical and sub tropical countries in the world. The edible portion is formed from swollen leaf sheaths derive from bladed leaves and the inner ones are bulb scales which is known as bulb and develop underground (Brewster, 1994) [2]. On the basis of skin colour there are three types of onion i.e., Red, Yellow and White. The red colour of onion is due to pigment ‘Anthocyanin’ and yellow colour is due to ‘Quercetin’ pigment. A volatile oil known as “Allyl-propyl disulphide” is the main ingredient responsible for pungency and flavor in bulbs, which help to prevent cancer and acts as a gastric stimulant and promotes digestion (Yawalkar, 1992) [12]. Chopping an onion causes damage to cells which allows enzymes called aliinases to break down amino acid sulfoxides and generate sulfenic acids through lachrymatory factor synthase (LFS) and giving volatile gas known as the onion lachrymatory factor or LF. Tear glands produces tears in order to dilute and flush out the irritant (Imai *et al.*, 2002) [3]. The productivity of the onion in India is low as compared to many other countries. This is mainly due to many fungal, bacterial and viral diseases. They are responsible for limiting the production and productivity of onion. Among the foliar diseases, *Stemphylium vesicarium*, the causal agent of white blotch of onion are being considered as an organism involved indirectly with the development of purple blotch (*Alternaria porri*) of onion. It is considered that *S. vesicarium* initiate the infection, which facilitates subsequent infection of *A. porri* causing purple blotch and hence the disease is designated as foliar blight complex (Zakirul, 2013) [13].

2. Material and methods**2.1. Collection and isolation of diseased samples**

The diseased samples of onion showing typical leaf blight symptoms were collected from Horticultural farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar Gujarat, India. The infected samples were brought to the laboratory and subjected for tissue isolation on to sterilized Potato dextrose agar (PDA) medium in Petri-plates.

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The Petri-plates were incubated at temperature 25 + 2 Search Results Featured snippet from the web °C for seven days. The culture was purified through dilution method and hyphal tip method.

2.2. Poisoned food technique

The effect of fungicides on combined mycelial growth of *A. porri* and *S. vesicarium* (both) pathogens were tested by poisoned food technique (Nene and Thapliyal, 2000) [7]. The required quantity of each test fungicide was added in conical flask containing 100 ml molten PDA medium so as to get required concentration in ppm. The flask containing poisoned medium was well shaken to facilitate uniform mixture of fungicide and 20 ml was poured in sterilized Petri-plates. On solidification of the medium, the plates were inoculated with seven mm disc of mycelial bit taken from the periphery of seven days old culture with the help of cork borer. The inoculated Petri-plates were incubated at 25 ± 2 °C. Three Petri-plates were used for each treatment. A Petri-plate without fungicide was served as control.

The experiment was conducted using factorial completely randomized design (FCRD) and data was statistically analysed using Duncan's New Multiple Range Test. Colony diameter was measured along the two diagonals passing through the colony by excluding the initial diameter (7 mm) of bit. Colony diameter was measured when the control treatment with pathogen reached full growth. The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by the following equation (Bliss, 1934) [1].

$$PGI = \frac{C - T}{C} \times 100$$

Where,

PGI=Per cent growth inhibition,

C=Colony diameter (mm) in control

T=Colony diameter (mm) in treatment

3. Results and discussion

3.1. Efficacy of systemic fungicides against associated pathogen(s) in vitro

The inhibitory effect of widely utilized different systemic fungicides were investigated against combined growth of *A. porri* and *S. vesicarium* in vitro and the results are presented in Table 1.

3.1.1. Effect of fungicides

All the five systemic fungicides were found inhibitory mean growth inhibition was ranged 28.75 to 84.07 per cent. The highest per cent growth inhibition (84.07%) recorded in tebuconazole was at par with propiconazole in which 81.94 per cent was recorded and it was closely followed by difenoconazole (80.51%). Hexaconazole (60.51%) was next best fungicide. While carbendazim recorded least mean growth inhibition and thereby proved less effective.

3.1.2. Effect of concentration

Irrespective of the fungicides, the inhibitory effect due to concentrations was increased positively with increasing concentration. The inhibition in radial growth was ranged 53.18 to 79.48 per cent at different concentrations.

Table 1: Growth inhibition of combined culture of *A. porri* and *S. vesicarium* by systemic fungicides at different concentration in vitro

S. No.	Fungicides	Growth inhibition (%)*				Mean
		Concentration (ppm)				
		50	100	250	500	
1	Carbendazim 50 WP	26.67 ^{**m} (20.18)	29.56 ^l (24.44)	34.80 ^k (32.59)	37.91 ^j (37.78)	32.24 ^d (28.75)
2	Tebuconazole 25.9 EC	57.58 ^g (71.29)	67.64 ^{de} (85.56)	69.53 ^{cd} (87.78)	73.20 ^b (91.67)	66.99 ^a (84.07)
3	Difenoconazole 25 EC	57.24 ^g (70.74)	63.82 ^f (80.56)	66.76 ^c (84.45)	68.27 ^{de} (86.30)	64.02 ^b (80.51)
4	Hexaconazole 5 EC	43.93 ⁱ (48.15)	47.11 ^h (53.70)	46.68 ^h (52.96)	69.08 ^{cde} (87.22)	51.70 ^c (60.51)
5	Propiconazole 25 EC	48.17 ^h (55.56)	69.35 ^{cde} (87.59)	71.72 ^{bc} (90.18)	76.38 ^a (94.44)	66.41 ^a (81.94)
	Mean	46.72 ^d (53.18)	55.50 ^c (66.37)	57.90 ^b (69.59)	64.97 ^a (79.48)	-
		Fungicide		Concentration	Fungicide × Concentration	
	S. Em. ±	0.39		0.35	0.79	
	C.V.%	2.42				

* Average of three observations;
 ** Arc-sin transformed values
 Figures in parentheses are original values.
 Treatment means with the letter(s) in common are not significant by Duncan's New Multiple Range test at 5 per cent level of significance

3.1.3 Interaction effect of fungicide × concentration

The interaction effect of fungicide × concentration was also found significant. More than 90 per cent growth inhibition was recorded in propiconazole and tebuconazole (94.44 and 91.67%, respectively) at 500 ppm concentration. The next best in order of merit was propiconazole (90.18%) at 250 ppm concentration. At lowest concentration of 50 ppm, tebuconazole and difenoconazole recorded more than 70 per cent growth inhibition (71.29 and 70.24%, respectively). Carbendazim (37.78%) was less effective inhibition even at highest concentration of 500 ppm. Tebuconazole, propiconazole and difenoconazole recorded more than 80% growth inhibition at 100 and 250 ppm while hexaconazole (87.22%) was found effective only at higher concentration of 500 ppm. Thus, it is evident from the data that propiconazole

at 500 ppm was significantly superior to rest of the combinations and it was closely followed by tebuconazole at the same concentration, however it was at par the propiconazole at 250 ppm concentration.

In the present study, tebuconazole and propiconazole among systemic fungicides were proved highly effective against combined growth of *A. porri* and *S. vesicarium*. In these experiment, benzimidazole and triazole group of fungicides have been used. Benzimidazole fungicide acts as a spindle poison binding to the protein subunits of spindle microtubules. That inhibits mitosis as described by Singh (2018) [9]. While, Triazole fungicides are Sterol biosynthesis inhibiting fungicides. Ergosterol is a cellular component that plays a crucial role in the structure and function of cell wall as described by Singh (2018) [9]. In the present study also,

triazole fungicide proved their superiority in inhibiting the fungal growth (Fig. 1).

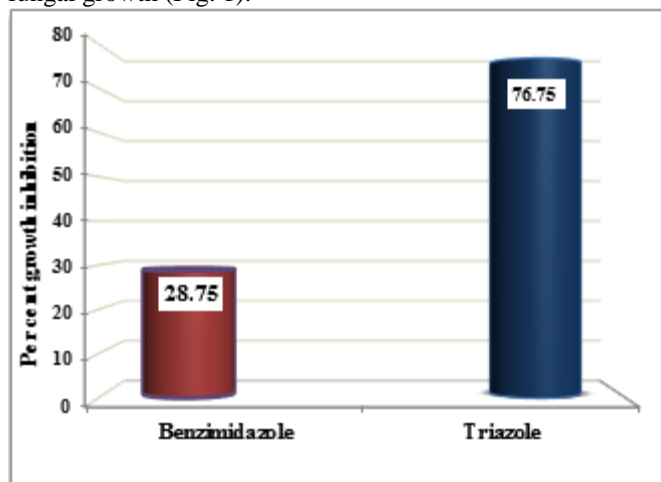


Fig 1: Efficacy of different group of fungicide against combined growth of *Alternaria porri* and *Stemphylium vesicarium*

Thaware (2014) reported that propiconazole inhibited 100 and 94.56 per cent inhibition in the growth of *A.alternata* and *S. vesicarium* (Mishra and Singh, 2017) [5]. Among the systemic fungicides tested, triazole fungicides viz., tebuconazole and difenoconazole proved superior in inhibiting growth of *A. porri* and *S. vesicarium*, respectively under *in vitro* conditions, as described by Yadav *et al.*(2017)[11]. Difenoconazole at 250, 500, 1000 and 2000 ppm and tebuconazole at 1000 and 2000 ppm concentration completely inhibited the mycelial growth of *A. porri* followed by

tebuconazole at 250 and 500 ppm with 86.67 and 88.89 per cent growth inhibition, respectively (Jhala *et al.*, 2017)[4].

Thus, the results obtained in present study confirm the findings of earlier reports.

3.2. Efficacy of non-systemic fungicide against associated pathogen(s) *in vitro*

The inhibitory effects of widely utilized different non-systemic fungicides were investigated against combined growth of *A. porri* and *S. vesicarium in vitro* and the results are presented in Table 2.

3.2.1. Effect of fungicides

All the five non-systemic fungicides were found inhibitory mean growth inhibition was ranged 30.56 to 79.07 per cent. The highest per cent growth inhibition (79.07%) recorded in propineb was at par with copper oxychloride (75.60%) was recorded and it was closely followed by captan and mancozeb (60.60 and 58.98%, respectively), and both were at par with each other. While chlorothalonil recorded least mean growth inhibition and thereby proved less effective.

3.2.2. Effect of concentration

Irrespective of the fungicides, the inhibitory effect due to concentrations was increased positively with the increasing concentration. The inhibition in radial growth was ranged 52.74 to 69.85 per cent at different concentrations. High per cent growth inhibition (69.85%) was observed at the higher concentration of 2000 ppm and it was significantly superior to rest of the concentrations.

Table 2: Growth inhibition of combined culture of *A. porri* and *S. vesicarium* by non systemic fungicides at different concentration *in vitro*

S. No.	Fungicides	Growth inhibition (%)*				Mean
		Concentration (ppm)				
		500	1000	1500	2000	
1	Copper oxychloride 50WP	59.90** ^{acd} (64.07)	59.61 ^{cd} (74.26)	61.86 ^{bc} (79.26)	65.91 ^a (84.81)	61.57 ^a (75.60)
2	Mancozeb 75WP	45.62 ⁱ (51.11)	48.17 ^{ghi} (55.56)	50.54 ^{fg} (59.63)	56.54 ^c (69.63)	50.22 ^b (58.98)
3	Chlorothalonil 75WP	27.84 ^l (21.85)	33.84 ^k (29.63)	35.67 ^k (33.33)	38.76 ^l (37.41)	34.03 ^c (30.56)
4	Propineb 70WP	59.37 ^{cde} (74.07)	61.74 ^{bc} (77.22)	64.49 ^{ab} (80.37)	66.91 ^a (84.63)	63.13 ^a (79.07)
5	Captan 75 WP	46.89 ^{hi} (52.59)	48.81 ^{sh} (56.67)	51.86 ^f (60.37)	58.53 ^{de} (72.78)	51.52 ^b (60.60)
	Mean	47.72 ^d (52.74)	50.43 ^c (58.67)	52.88 ^b (62.59)	57.33 ^a (69.85)	-
		Fungicide		Concentration		Fungicide × Concentration
	S. Em. ±	0.43		0.39		0.86
	C.V.%			2.86		

* Average of three observations;

** Arc-sin transformed values

Figures in parentheses are original values.

Treatment means with the letter(s) in common are not significant by Duncan's New Multiple Range test at 5 per cent level of significance

3.2.3. Interaction effect of fungicide × concentration

The interaction effect of fungicide × concentration was also found significant. More than 80% growth inhibition was recorded in copper oxychloride and propineb (84.61 and 84.63%, respectively) at 2000 ppm concentration. The next best in order of merit was propineb at 1500 ppm concentration with 80.37% growth inhibition. At lowest concentration of 500 ppm, propineb and copper oxychloride recorded more than 60% growth inhibition (74.07 and 64.07%, respectively). Chlorothalonil was less effective as it recorded only 37.41%

growth inhibition even at highest concentration of 2000 ppm. Copper oxychloride and propineb recorded more than 70% growth inhibition at 1000 and 1500 ppm and mancozeb and captan recorded more than 50% growth inhibition at 500 and 1000 ppm concentration. While, captan (72.78%) was found effective only at higher concentration of 2000 ppm. Thus, it is evident from the data that propineb at 2000 ppm was significantly superior to rest of the combinations and it was closely followed by copper oxychloride at the same concentration and both were at par with each other (Fig. 2).

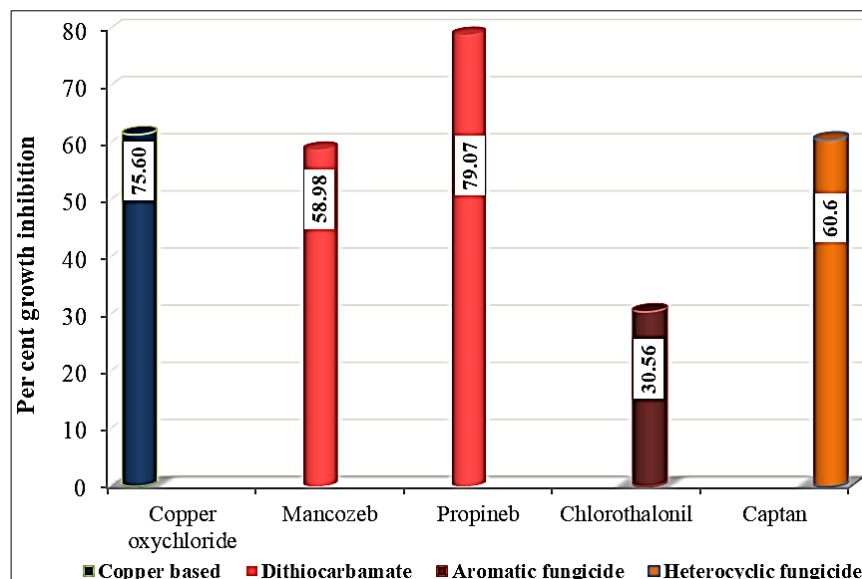


Fig 2: Per cent growth inhibition of *A. porri* and *S. vesicarium* by non-systemic fungicides at different concentration *in vitro*

Different non-systemic fungicides against the fungus *Alternaria alternata* under *in vitro* condition were evaluated by Thaware *et al.* (2011) [10] and reported mancozeb (0.2%) as highly effective against the growth of the *Alternaria alternata*. Priya (2014) [8] reported that mancozeb 75 WP and propineb 70 WP were highly effective against the mycelial growth of *A. porri* and both were at par with each other followed by chlorothalonil.

Thus, the results obtained in present investigation are more or less in accordance with the results of earlier reports.

3.3. Efficacy of compound fungicide against associated pathogen *in vitro*

The inhibitory effects of widely utilized different compound fungicides were investigated against combined growth of *A. porri* and *S. vesicarium* *in vitro* and the results are presented in Table 3.

3.3.1. Effect of fungicides

All the four compound fungicides were found inhibitory to

the combined radial growth of *A. porri* and *S. vesicarium*. The mean growth inhibition was ranged 45.83% to 83.33%. The highest per cent growth inhibition (89.07%) was recorded in tebuconazole 50 WG + trifloxistrobin 25 WG which was significantly superior to rest of the compound fungicides. The next best in order of merit was carboxin 37.5 DS + thiram 37.5 DS (60.97%) was at par with carbendazim 12 WP + mancozeb 63 WP with 62.78% growth inhibition, while metalaxyl 8 WP + mancozeb 64 WP recorded least mean growth inhibition (45.83%) and thereby proved less effective.

3.3.2 Effect of concentration

Irrespective of the fungicides, the inhibitory effect due to concentrations was increased positively with the increasing concentration. The inhibition in radial growth was ranged 48.70 to 77.18 per cent at different concentrations. High per cent growth inhibition (77.18%) was observed at the higher concentration of 500 ppm and it was significantly superior to rest of the concentrations.

Table 3: Growth inhibition of combined culture of *A. porri* and *S. vesicarium* by compound fungicides at different concentration *in vitro*

S. No.	Fungicides	Growth inhibition (%)*				Mean
		Concentration (ppm)				
		50	100	250	500	
1	Tebuconazole 50 WG + Trifloxistrobin 25 WG	60.73 ^{***} (76.11)	64.50 ^b (81.48)	68.92 ^a (86.67)	70.68 ^a (89.07)	66.21 ^a (83.33)
2	Metalaxyl 8 WP + Mancozeb 64 WP	34.4 ^b (32.04)	39.75 ^e (40.93)	45.72 ^f (51.30)	50.54 ^d (59.08)	42.62 ^c (45.83)
3	Carboxin 37.5 DS + Thiram 37.5 DS	33.53 ^h (30.55)	46.68 ^{ef} (52.96)	61.71 ^b (74.82)	70.17 ^a (85.56)	53.02 ^b (60.97)
4	Carbendazim 12 WP + Mancozeb 63 WP	48.92 ^{de} (56.11)	49.24 ^{de} (58.15)	51.84 ^d (61.85)	60.00 ^c (75.00)	52.50 ^b (62.78)
	Mean	44.41 ^d (48.70)	50.04 ^c (58.38)	57.04 ^b (68.66)	62.85 ^a (77.18)	-
		Fungicide		Concentration		Fungicide × Concentration
	S. Em. ±	0.46		0.46		0.92
	C.V.%	2.98				

*Average of three observations;

** Arc-sin transformed values

Figures in parentheses are original values.

Treatment means with the letter(s) in common are not significant by Duncan's New Multiple Range test at 5 per cent level of significance

3.3.3 Interaction effect of fungicide × concentration

The interaction effect of fungicide × concentration was also found significant. More than 85% growth inhibition was recorded in tebuconazole 50 WG + trifloxistrobin 25 WG and carboxin 37.5 DS + thiram 37.5 DS (89.07 and 85.56%, respectively) at 500 ppm concentration. The next best in order

of merit was tebuconazole 50 WG + trifloxistrobin 25 WG at 250 ppm concentration with 86.67% growth inhibition and they all were at par with each other at lowest concentration of 50 ppm, tebuconazole 50 WG + trifloxistrobin 25 WG recorded more than 70% growth inhibition (76.11%). Metalaxyl 8 WP + mancozeb 64 WP (59.08%) was less

effective at highest concentration of 500 ppm. Carboxin 37.5 DS + thiram 37.5 DS and carbendazim 12 WP + mancozeb 63 WP recorded more than 60% growth inhibition at 250 ppm. Carbendazim 12 WP + mancozeb 63 WP (75.00%) at higher concentration of 500 ppm recorded growth inhibition of 75%. Thus, it is evident from the data that tebuconazole 50 WG + trifloxistrobin 25 WG at 500 ppm and carboxin 37.5 DS + thiram 37.5 at 500 ppm concentration and tebuconazole 50 WG + trifloxistrobin 25 WG at 250 ppm concentration were highly efficacious combinations as they recorded significantly highest inhibition of the combined growth of *A. porri* and *S. vesicarium*.

Mishra and Gupta (2012) [6] tested the efficacy of eight fungicides and reported that carbendazim 12 WP + mancozeb 63 WP was found effective in inhibiting the colony growth of *A. porri* and *S. vesicarium*. *In vitro* evaluation of different systemic fungicides revealed that tebuconazole 50 WG + trifloxistrobin 25 WG resulted in mean growth inhibition of 69.61% against *A. porri* (Yadav *et al.*, 2017) [11]. Evaluation of seven fungicides revealed that carboxin 37.5 DS + thiram 37.5 (0.3%) resulted in cent per cent mycelia growth inhibition of *A. porri* (Bhanderkar *et al.*, 2019).

Among compound fungicides, combination of tebuconazole 50 WG + trifloxistrobin 25 WG was proved most effective in inhibiting the growth of the fungus. This might be due to the fact that tebuconazole is a narrow spectrum and trifloxistrobin is a broad spectrum and thus balanced fungicide. Tebuconazole is a triazole group fungicide that acts as a sterol biosynthesis inhibitor and trifloxistrobin is a strobilurin group of fungicide that acts as a mitochondrial electron transport inhibitors.

Thus, it proved its superiority in inhibiting the combined mycelial growth of *A. porri* and *S. vesicarium* in present study also.

Thus, it can be inferred from the above findings or effectiveness of various systemic (narrow spectrum) and non-systemic (broad spectrum) that narrow spectrum fungicides are more site specific in inhibiting the pathogen and this proved more efficacious than broad spectrum. The mean inhibition in growth with narrow spectrum fungicide was 67.16% compared to 60.69% in broad spectrum (Fig 3).

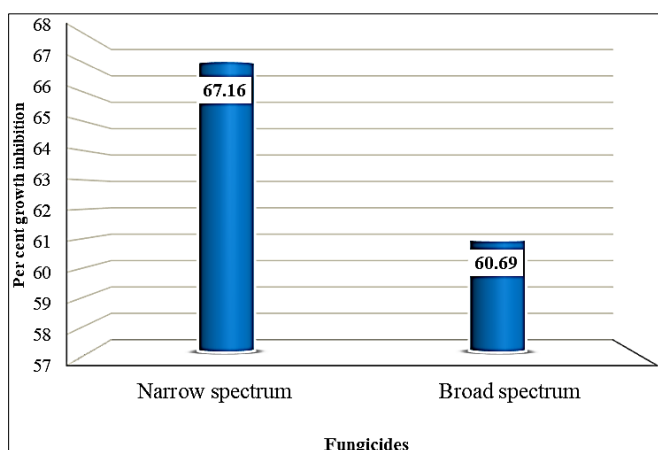


Fig 3: Efficacy of systemic (Narrow spectrum) and non-systemic (Broad spectrum) fungicide against *A. porri* and *S. vesicarium*

4. Acknowledgement

The authors humbly acknowledge the Director of Research and Dean (PG) as well as Dean (Agri), Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada

Agricultural University, Sardarkrushinagar for the facilities and assistance.

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