The Pharma Innovation Journal 2022; 11(11): 1240-1245

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(11): 1240-1245

www.thepharmajournal.com
Received: 25-09-2022
Accepted: 30-10-2022

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In vitro bio-efficacy of botanical extracts and bioagents against *Ascochyta phaseolorum* causing cowpea Ascochyta blight in Meghalaya

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Abstract
Cowpea [*Vigna unguiculata* (L) Walp.] is a pulse crop utilised as vegetable source and fodder. Ascochyta leaf blight caused by *Ascochyta phaseolorum* under humid conditions is often devastating, causing extensive defoliation of cowpea. In this study, plant extracts and bio-control agents were evaluated *in vitro* against the growth of the pathogen. Among the plant extracts, *Solanum torvum* at 15% (82.33%), 10% (80.37%) and 5% (77.65%) were found effective against growth of *A. phaseolorum.* The *Azadirachta indica* extract at 15% (64.78%) and 10% (51.22%), *Curcuma longa* at 15% (51.37%), *Acorus calamus* 15% (48.77%) and *Aloe vera* at 15% (45.30%) were moderately effective. Extract of *W. somnifera* at 5% with16.51% percent inhibition recorded the least efficacy. Among the bio-control agents (BCAs), maximum percent inhibition was observed in *Trichoderma harzianum* (65.93%) which was significantly superior to the rest of BCAs tested and the next best antagonist was *T. viride* (60.86%). Bacterial antagonists *Pseudomonas fluorescens* showed moderate percent inhibition (45.31%) followed by *Bacillus subtilis* strain BS217 (38.64%) and least efficacy was observed in *B. subtilis* strain BC07 (25.56%). The findings provide the promising efficacy of readily available botanicals *viz. S. torvum* and *A. indica* on management of *A. phaseolorum* on cowpea. Further studies on *in-vivo* and field efficacy of the plant extracts are required.

Keywords: Cowpea, plant extracts, bio-control agent, *Ascochyta phaseolorum*

Introduction
Cowpea [*Vigna unguiculata* (L.) Walp.] is assumed to be the principal ancient pulse crop of India. Cowpea is an important grain legume mainly grown for its seed, as a vegetable crop, for fodder, green manure, as a cash crop and cover. Cowpea dry grain contains 23–32% protein and essential amino acids (Carvalho *et al*., 2017) [5]. Also, the cowpea fresh and immature pods, green seeds and leaves were utilised as vegetable sources (Gerrano *et al*., 2019) [6]. However, cowpea productivity is limited by a wide range of biotic stress factors, including destructive pests, fungal, bacterial and viral pathogens, as well as parasitic weeds (Boukar *et al*., 2019) [3]. Among many fungal diseases, ascochyta blight caused by *Ascochyta phaseolorum* is a major disease of cowpea and many other legumes under humid conditions often devastating, causing extensive defoliation (Singh and Allen, 2006) [18]. Ascochyta blight can lead to more than 50 percent or 75 percent of yield loss in pea (McDonald and Peck, 2009 and Salam *et al*., 2011) [11, 7]. Ascochyta blight disease has economic importance in the regions with cool humid condition. There are more reports on temperature, continuous rainfall and cloudy weather conditions during growing season which enhance the development and distribution of the disease (Pande *et al*., 2005) [15]. In order to obtain more and good quality yield, famers often rely on chemical crop protection method. However, chemical management methods are environmentally detrimental, adverse effects on human health and other animals, toxic to natural enemies and resistance developed by pathogen. Consequently, it is desirable to search for an alternative by using the natural biological balance to control the disease. Many scientists across the globe explored and experimentally validated different biopesticides and bio-control agents for managing different plant diseases. The antifungal activity of several plant extracts was reported (Khan *et al*., 2021; Rashid *et al*., 2015; Jargees *et al*., 2010) [21, 16, 7]. Campanella and Miceli, (2021) [4] through use of biocontrol agents on *Fusarium* wilt in lentils recorded a reduced disease incidence up to 50.0% and increased yield up to 58.7%.

Considering the potential role of biological control as an eco-friendly and alternate approach for disease management strategies, the present study was undertaken to check the efficacy of different plant extract and biocontrol agent *in vitro* against the growth of *A. phaseolorum* pathogen which cause Ascochyta leaf blight in cowpea.

Materials and Methods

Isolation and maintenance of *A. phaseolorum*

The leaves of cowpea showing typical leaf blight symptoms collected from farmer's field in Meghalaya were brought to the laboratory. Samples of infected cowpea leaves pieces were cut, rinsed and sterilized in 1% sodium hypochlorite (NaOCl) for one minute and three changes of sterile distilled water before being plated on Potato Dextrose Agar (PDA), prior to the incubation. Developing fungal colonies were purified by hyphal tip-cut method to obtain pure culture of the isolates. The pathogen was identified by comparing with the cultures in the laboratory and relevant literatures. It was further stored at about 4 ℃ before use.

In vitro efficacy of plant extracts against *A. phaseolorum*

Six locally available plants with defined antifungal properties were used in the present study. The plant and their parts such as leaves of *Azadirachta indica*, *Withania somnifera*, *Acorus calamus*, *Aloe vera*, *Withania somnifera* and *Solanum torvum* were used individually to prepare extracts.

Preparation of plant extracts

The aqueous plant extracts of the selected botanicals were obtained as per the method described by Bhatti (1988) [2]. A 100 g of sample of each plant was washed with distilled, sterile water. Then each sample was ground separately by using sterile pestle and mortal in 100 ml distilled sterile water. The extract of each sample thus obtained was filtered separately through a sterilized double layered muslin cloth to remove the bits of plant material is the filtrate. Then this extract was again filtered through a filter paper (Whatman No.1). The filtered extract was centrifuged at 4000 rpm for 5 minutes to get homogenous aqueous solution. The supernatant was sterilized finally through bacteria proof membrane syringe filter (0.22 µm) under laminar air flow. The final clear extracts prepared was the standard plant extracts of 100% concentration and were stored at 4ºC in refrigerator.

The effect of plant extracts on mycelial growth was studied by 'Poisoned Food Technique' (Nene and Thapliyal, 1979) [13]. An appropriate quantity of each plant extract (100%) was mixed thoroughly with autoclaved and cooled (40 ºC) PDA medium in conical flasks (250 ml cap.) to obtain desired concentrations (5, 10 and 15 percent). The plates containing PDA without any plant extract was maintained as untreated control. Upon solidification of PDA, all the plates were aseptically inoculated by placing in the centre a 5 mm mycelial disc obtained from a five-day old actively growing pure culture. All these plates were then incubated at 27±1 °C for 7 days. Three replications were maintained for each treatment.

The mean colony growth of the test fungus was recorded when the control plate achieved full growth. Percentage inhibition (I) of the pathogen was calculated by following the formula described by Vincent (1927) [19]:

$$\text{Inhibition percent (I)} = \frac{C - T}{C} \times 100$$

Where,
I = percent inhibition of mycelial growth,
C = Growth in control plate (cm) and
T = Growth in treated plate (cm)

In vitro efficacy of bio-control agent against *A. phaseolorum*

In order to study the antagonism of the bio-agents against the test pathogen, two fungal bio-agents (*Trichoderma harzianum* and *T. viride*) and three bacterial bio-agent (*Pseudomonas fluorescens*, *Bacillus subtilis* strain BS217 and *B. subtilis* strain BC07) were obtain from Plant Pathology Laboratory (PGSAS, Umiam). The experiment was carried out by dual culture technique (Padder *et al*., 2010) [14]. Fungal bioagents and the test pathogen were grown on PDA and the bacterial bio-agent was grown on nutrient agar medium. For dual culture technique, a 5 mm disc of five-day old actively growing culture of the pathogen was placed at 1.5 cm away from the edge of each Petri plate containing PDA medium. On the opposite of the pathogen, a 5 mm disc of the fungal antagonist was placed. PDA plates inoculated with the pathogen alone at the centre of the plate served as the control. For bacterial antagonists, a 5 mm disc of five-day old actively growing culture of the pathogen was inoculated at the centre of the Petri plate containing PDA medium and incubated at 27±1 °C. After 48 hours, one-day old actively growing culture of the test bacteria were streaked separately into the media at two margins of the plate *i.e*., 1.5 cm away from the edges. PDA plates inoculated with the pathogen alone at the centre of the plate served as the control. Three replications of each treatment were maintained. The observation on colony diameter of the fungus were recorded when Petri plate in control treatment was fully covered with mycelial growth. Percent inhibition of growth of the test pathogen was calculated as described above.

Results and Discussion

This study was carried out to check the *in-vitro* efficacy of six different plant aqueous extracts at different concentrations against mycelium growth of *A. phaseolorum* by following poisoned food technique (Plate 1). Further, *in-vitro* efficacy of five bio-control agents against mycelium growth of *A. phaseolorum* by dual culture technique was evaluated (Plate 2). The bar diagram of the results is presented in Fig. 1 and 2. The percent inhibition was worked out when the mycelium growth of the pathogen in control plate covers full growth. The data obtained were statistically analysed and presented in Table 1 and 2.

Table 1: *In vitro* efficacy of plant extracts against mycelium growth of *A. phaseolorum*

Treatments	Mean colony diameter (cm)			Percent inhibition		
	5%	10%	15%	5%	10%	15%
A. indica	5.13±0.78 (2.26)	4.39±0.51 (2.09)	3.17±0.44 (1.78)	43±1.34 (6.56)	51.22±0.98 (7.16)	64.78±1.1 (8.05)
W. somnifera	7.51±0.02 (2.74)	7.09±0.02 (2.66)	5.05±0.03 (2.24)	16.51±0.31 (4.06)	21.14±0.16 (4.60)	43.88±0.33 (6.62)
A. calamus	7.21±0.05 (2.68)	5.62±0.03 (2.37)	4.61±0.04 (2.14)	19.89±0.65 (4.45)	37.52±0.33 (6.12)	48.77±0.44 (6.98)
A. vera	7.46±0.02 (2.73)	5.59±0.04 (2.36)	4.92±0.11 (2.21)	17.16±0.32 (4.14)	37.90±0.53 (6.15)	45.30±1.30 (6.73)
C. longa	7.12±0.09 (2.66)	6.83±0.03 (2.61)	4.38±0.06 (2.09)	20.86±1.01 (4.56)	24.07±0.42 (4.90)	51.37±0.69 (7.16)
S. torvum	2.01±0.04 (1.41)	1.77±0.03 (1.33)	1.59±0.03 (1.24)	77.65±0.44 (8.81)	80.37±0.37 (8.96)	82.33±0.44 (9.07)
Control	9.00±0.00 (3.00)	9.00±0.00 (3.00)	9.00±0.00 (3.00)	0.00±0.00 (0.70)	0.00±0.00 (0.70)	0.00±0.00 (0.70)
CD (p=0.05)	2.10	1.64	2.67	0.19	0.15	0.24

(**Note:** Values within the parentheses are square root transformed values)

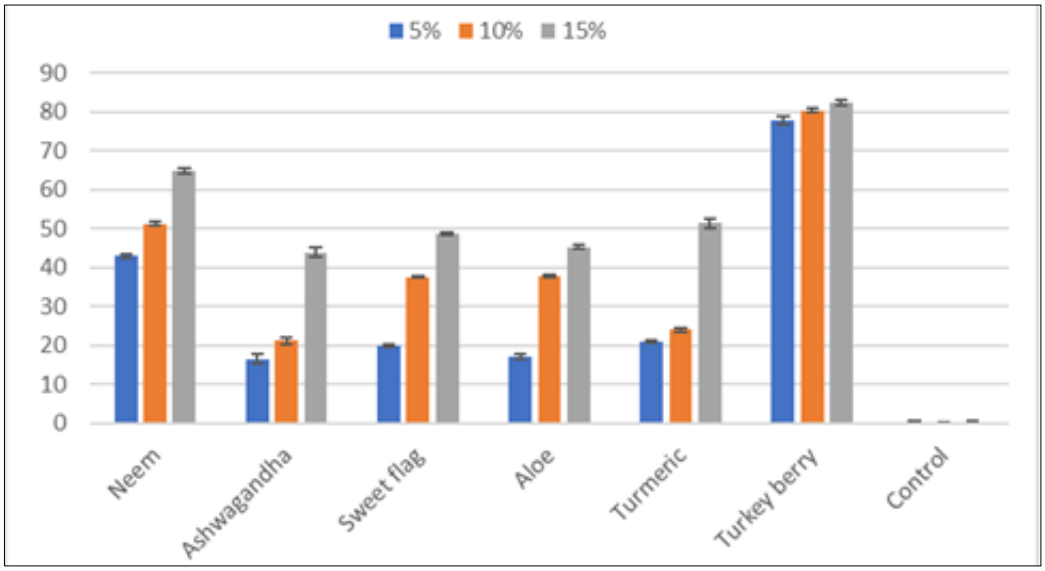
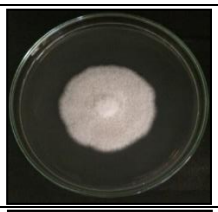
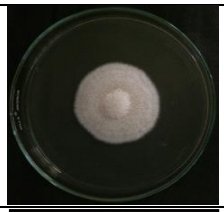
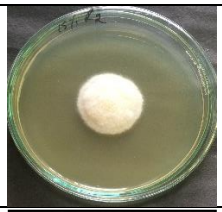
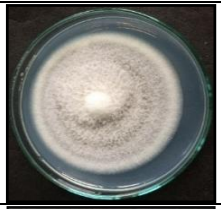
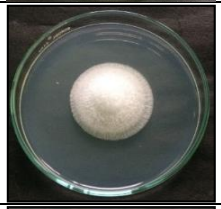
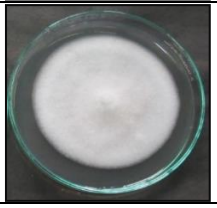
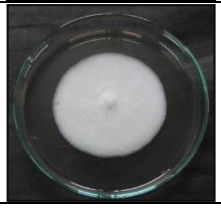
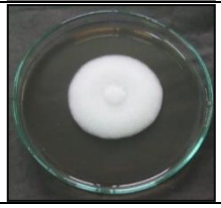


Fig 1: Bar diagram showing *in vitro* efficacy of plant extracts against mycelial growth of *A. phaseolorum*

	5%	10%	15%
A. indica			
W. somnifera			
A. calamus			

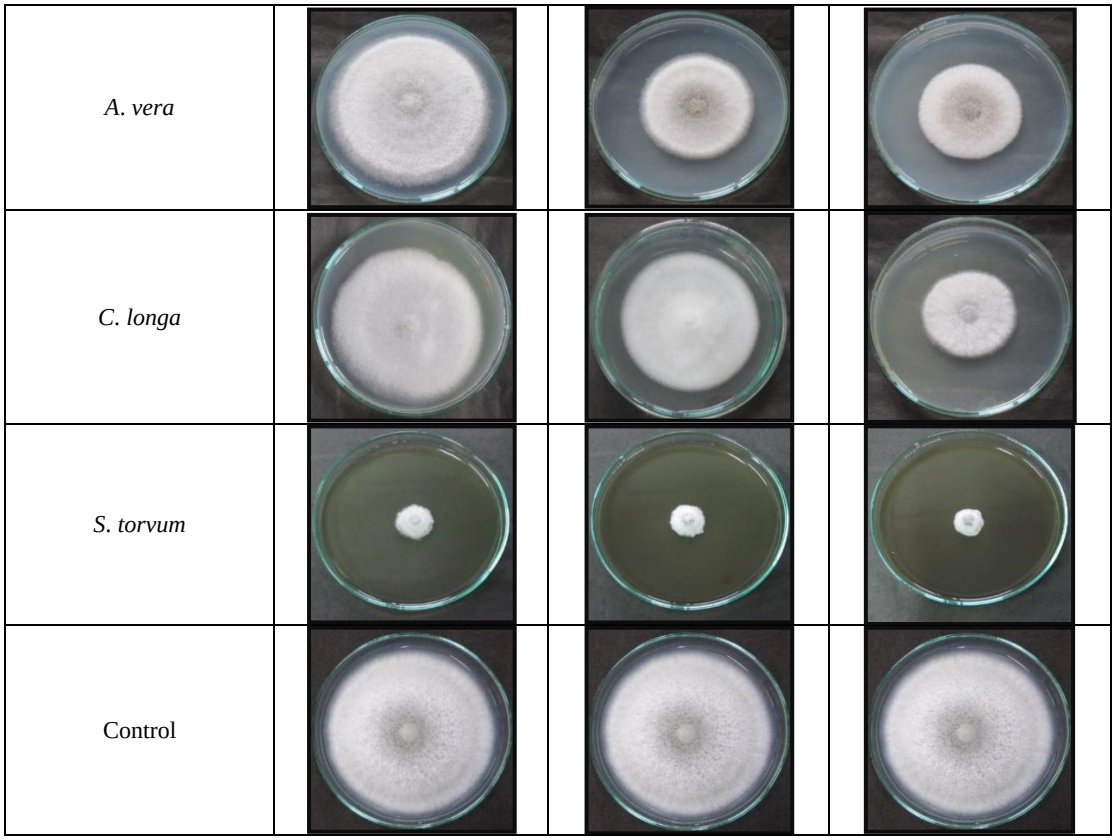


Plate 1: *In vitro* efficacy of plant extracts at different concentrations on mycelial growth of *A. phaseolorum*

Table 2: *In vitro* efficacy of bio-control agents against mycelial growth of *A. phaseolorum*

BCAs	Growth (cm)	Percent Inhibition
T. viride	3.52^{e}±0.02 (1.87)	60.86^{b}±0.24 (7.81)
T. harzianum	3.07^{f}±0.03 (1.75)	65.93^{a}±0.37 (8.12)
P. fluorescens	4.92^{d}±0.06 (2.21)	45.31^{c}±0.75 (6.73)
B. subtilis BS217	5.52^{c}±0.04 (2.35)	38.64^{d}±0.44 (6.22)
B. subtilis BC07	6.70^{b} ± 0.03 (2.58)	25.56^{e}±0.47 (5.05)
Control	9.00^{a}±0.00 (3.00)	0.00^{f}±0.00 (0.70)
CD (p=0.05)	0.02	0.10

(**Note:** Values within the parentheses are square root transformed values)

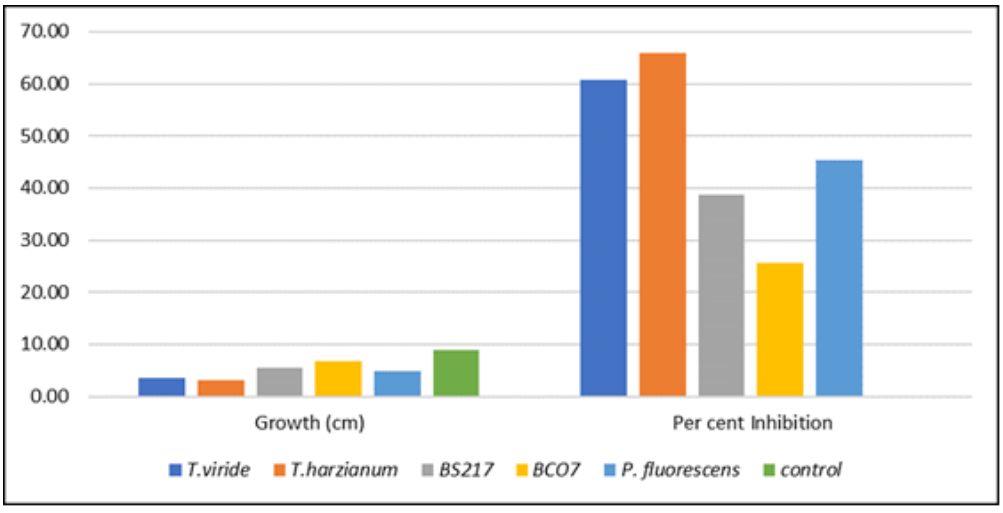


Fig 2: Bar diagram showing *in vitro* efficacy of bio-control agents against mycelial growth of *A. phaseolorum*

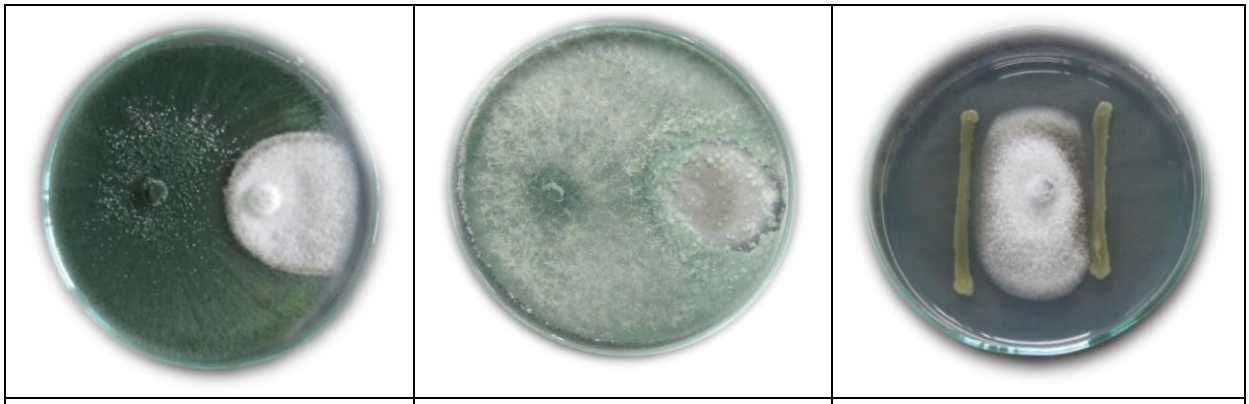


Plate 2: *In vitro* efficacy of bio-control agents against mycelial growth of *A. phaseolorum*

Out of the six plant extracts evaluated against the growth of *A. phaseolorum*, aqueous leaf extracts of *S. torvum* at 15% (82.33%), 10% (80.37%) and 5% (77.65%) were found effective against growth of *A. phaseolorum*. The *A. indica extract* at 15% (64.78%) and 10% (51.22%), *C. longa* 15% (51.37%), *A. calamus* 15% and *A. vera* 15% (45.30%) were moderately effective while rest of the plant extracts were comparatively less effective. The efficacy of *S. torvum* for its antifungal potential against *A. phaseolorum* was followed by *A. indica* extracts. The present finding is in par with Lalitha *et al*. (2010) [9]. They reported that aqueous extract of leaves of *S. torvum* at 25% concentration could inhibit 100% on growth of *Pyricularia grisea*. Such report of *S. torvum* efficacy has not been reported on cowpea by any workers till date. The effectiveness of *S. torvum* might be due to the presence of alkaloids. Naseer *et al*. (2022) [12] also reported colony growth inhibition of 50.3% against *Ascochyta rabiei* by *A. indica* extracts. The antifungal activity of *A. indica* plant extract (propyl disulfide) could be attributed to the sulfur compound as sulfur compounds are well-known for microbial growth prevention (Khan *et al*., 2021) [16]. Among the five BCAs were evaluated for their antagonistic potential on the mycelial growth of *A. phaseolorum* using dual culture technique, maximum percent inhibition was observed in *T. harzianum* (65.93%) which was significantly superior to the rest of BCAs tested and the next best antagonist was *T. viride* (60.86%). The results obtained were supported by findings of Benzohra (2011) [1] that mycelial growth of *A. rabiei* was significantly inhibited by *T. harzianum* with a creation of a zone of inhibition. It was further observed that among the bacterial antagonists *P. fluorescens* showed highest percent inhibition (45.31%) followed by *B. subtilis* strain BS217 (38.64%) and least efficacy was observed in *B. subtilis* strain BC07 (25.56%). Liu *et al*. (2016) [10] reported that *Bacillus* sp. and *Pantoea agglomerans* can control *A. pinodes* causing ascochyta blight in field peas.

Conclusion

The present study revealed the mycelium growth inhibition potential of plant extracts and bio-agents against *A. phaseolorum*. Based on *in vitro* experiment, plant extracts *viz*., *S. torvum* and *A. indica* bio-control agent *viz*., *T. harzianum* and *T. viridi* exhibited significant high efficacy against the mycelium growth of *A. phaseolorum*. Additionally, plant extracts (*W. somnifera, A. calamus, C. longa and A. vera*) and control agent (*P. fluorescens* and *B. subtilis*) were associated with moderate reduction of mycelium growth of *A. phaseolorum*. These findings may be further study under *in vivo* experiments to profile the efficacy for integrated disease management for ascochyta blight.

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