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Evaluation of Bioagents against *Fusarium oxysporum* f. sp. *pisi* Causing Wilt Disease in Pea

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ABSTRACT

In this study, *in vitro* potential of six selected species of *Trichoderma*, including *Trichoderma viride*, *Trichoderma virens*, *Trichoderma harzianum, Trichoderma longibrachiatum, Trichoderma ressei* and *Trichoderma asperellum* as well as the of two bacterial isolates *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against *Fusarium oxysporum* f. sp. *pisi* causing wilt of pea. *Trichoderma virens* recorded maximum percent growth inhibition (81.97%). In volatile compounds percent growth inhibition 86.80 % was recorded highest in *Trichoderma harzianum* and percent growth inhibition in non-volatile compound will increase as concentration of bioagents increases and 100% growth inhibition was recorded in *Trichoderma viride*, *Trichoderma virens*, *Trichoderma harzianum* and in *Trichoderma ressei* at 15% concentration.

Keywords *Bacillus*, *Fusarium*, *Pseudomonas*, *Trichoderma*, Wilt.

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INTRODUCTION

A major cash crop for farmers in Madhya Pradesh is the *rabi* pea (*Pisum sativum*). Numerous bacterial, fungal, and viral diseases affect this crop, and under the appropriate condition, diseases can greatly reduce both productivity and quality. The crop production is lower than what is possible due to biotic and abiotic stress. An economically significant disease, Fusarium wilt results in losses of between 30 and 40% (Gupta and Gupta 2019). In plants wilt symptoms are more prominent at 3 to 5 week old plants. Cotyledons droop and wither in early seedlings. *Fusarium oxysporum* f. sp. *pisi* is soil borne and is capable of saprophytic survival on crop residues in the soil for up to eight years. Because the required large-scale soil application of chemicals is costly, dangerous, and disturbs the biological balance, chemical control of the disease is consequently difficult, impractical, and uneconomical. Hence, efforts have to be made to curtail pathogen activity and restricting losses below economic threshold level by choosing alternative methods. Of late biocontrol methods involving manipulation of antagonistic rhizosphere microflora such as *Trichoderma, Pseudomonas* or *Bacillus* against soil borne plant pathogens. Certain species of *Bacillus* and *Pseudomonas* can be used as plant growth-promoting bacteria and biological control agents for several plant pathogens, as they are harmless to humans and animals while also being eco-friendly (Rathore *et al.* 2020). Recently *Trichoderma* is widely used as seed treatment and soil mix that promote the root growth and protect plant from infection of soil borne fungal pathogens. Considering economic importance of disease investigation was carried out to eco-friendly management (*in vitro*) of wilt disease of pea causing *Fusarium oxysporum* f. sp. *pisi*.

MATERIALS AND METHODS

Isolation and purification of the pathogen

Small piece of infected tissues 1-2 mm in size from the propelling edge of the wilted root, with healthy segments were cut with sharp blade, washed well in distilled water to take out dust stuck to the tainted pieces. Pieces were pre-treated 1% NaOCl (*Sodium hypochlorite*) for 1 minute and consequently washed well in three changes of disinfected refined water. The pieces were then moved to PDA test tube with the help of inoculating needle under aseptic condition and incubated at 28 ± 1°C. After 72 hrs, fragments of hyphal growth from the growing tips were moved to new PDA test tube. Pure culture was made, following rehashed hyphal tip transfer method.

Effect of bioagents on growth and sporulation of *Fusarium oxysporum*

For screening of the main antagonist against *Fusarium oxysporum* dual culture strategy created by Morton and Straube (1955) was taken on. Twenty ml sterilized liquefied PDA medium was filled into sanitized petriplates @ 20 ml/plate aseptically permitted to solidify, then, at that point, then 5 mm discs of the target pathogen and the antagonistic cut with the help of sterilized cork borer were put on PDA around 4 cm separated one another incubated in BOD incubator at 28 ± 1°C. Three replications were kept up with for every treatment. Observation on radial growth of bioagents and test pathogen was recorded after 96 hrs of incubation. Inhibition of mycelial growth and production of spore of target pathogen over check was calculated by following formula (Vincent 1947). In order to study the viability of target pathogen, isolation was done by transferring 5 mm mycelial disc cut by cork borer from the zone where the test fungus was already overgrown by the antagonist on PDA medium.

Effect of volatile compounds from biocontrol agents on radial growth and sporulation of *F. oxysporum*

The effects of volatile compounds from all bioagents on radial growth of *Fusarium oxysporum* were studied as per the method given by Dennis and Webster (1971). The two bottom portion of petriplates containing PDA were inoculated with a 5 mm disc of pathogen and other and sealed with cellophane adhesive tape and incubated in BOD incubator at 28 ±1°C. The petriplate containing PDA without antagonist serves as control. The observations on the radial growth of the test fungus were recorded after 120 hrs of incubation at 28 ± 1°C. The radial growth of the test fungus in the treatment in comparison with that of check gave percent growth inhibition.

Efficacy of non-volatile compounds from biocontrol agents radial growth and sporulation of *F. oxysporum*

The biocontrol agents were grown in *Potato dextrose* broth at 27°C with irregular shaking at 150 rpm. The metabolites were collected after 15 days and filtered. The sterilized filtrate was amended in PDA to make 5, 10 and 15% concentration in petriplates. The solidified agar plates in sets of three were inoculated at the middle with 5 mm diameter mycelial disc of target pathogen and incubated at 28 ± 1°C for 96 hrs. The plates without filtrate used as control. The colony diameter was recorded and percent inhibition of radial growth was determined utilizing the formula given by Vincent (1947). The statistical method used to conduct the experiments was in Completely Randomized Design method, during 2020-2021.

RESULTS AND DISCUSSION

Evaluation of antagonistic efficacy of bioagents

Bioagents, including fungi and bacteria, were tested for their effects on radial growth and sporulation of the test pathogen *Fusarium oxysporum* f. sp. *pisi*. Data on radial growth, percent growth inhibition and sporulation after 124 hrs of incubation are also shown in Table 1 and Fig. 1. The most effective pathogen was *Trichoderma virens* (T_2), with the highest rate of growth inhibition (81.97%), *Trichoderma reesei*

Table 1. Antagonistic efficacy of bioagents under dual culture technique and their volatile compounds against *Fusarium oxysporum* f. sp. *pisi* after 124 hrs of incubation.

	Treatment	Dual culture			Volatile compounds		
		Radial growth of pathogen	% growth inhibition	Sporulation	Radial growth of pathogen	% growth inhibition	Sporulation
T_1	*Trichoderma viride*	31.70	54.27	++	26.45	63.45	++
T_2	*Trichoderma virens*	12.50	81.97	_	20.31	71.93	+
T_3	*Trichoderma harzianum*	29.60	57.30	_	9.55	86.80	-
T_4	*Pseudomonas fluorescens*	38.40	44.60	++	31.16	56.95	+
T_5	*Bacillus subtilis*	45.20	34.79	++	30.88	57.34	+
T_6	*Trichoderma longibrachiatum*	30.10	56.57	-	31.73	56.15	+
T_7	*Trichoderma ressei*	25.20	63.64	_	27.78	61.61	+
T_8	*Trichoderma asperellum*	31.22	54.96	_	34.00	53.03	++
T_9	Control	69.31	0.00		72.37	0.00	++++
	SE (m)	**0.62**			**0.68**		
	CD (0.5)	**1.86**			**2.05**		

(T_7 - 63.64%), *Trichoderma longibrachiatum* (T_6 - 56.57%), *Trichoderma halzianum* (T_3 - 57.30%). The least effective pathogen was *Bacillus subtilis* (T_5), inhibited by only 34.79%, followed by *Pseudomonas fluorescens* (T_4 - 44.60%) and *Trichoderma viride* (T_1 - 54.27%). Among them, sporulation was

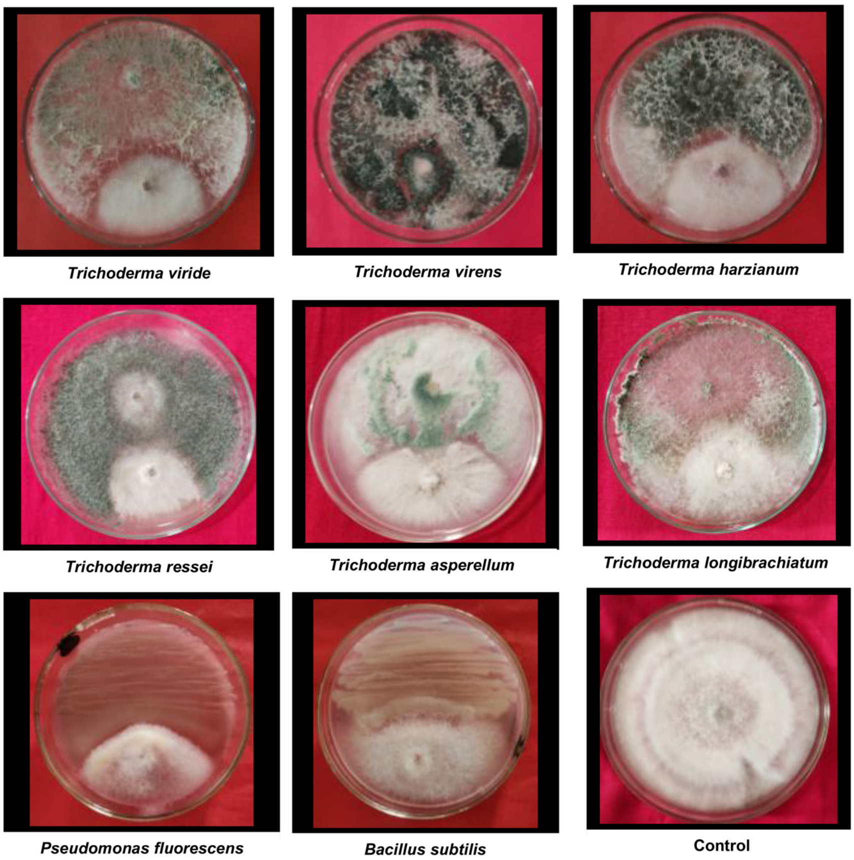

Fig. 1. Antagonistic efficacy of bioagents against *Fusarium oxysporum* by using dual culture technique.

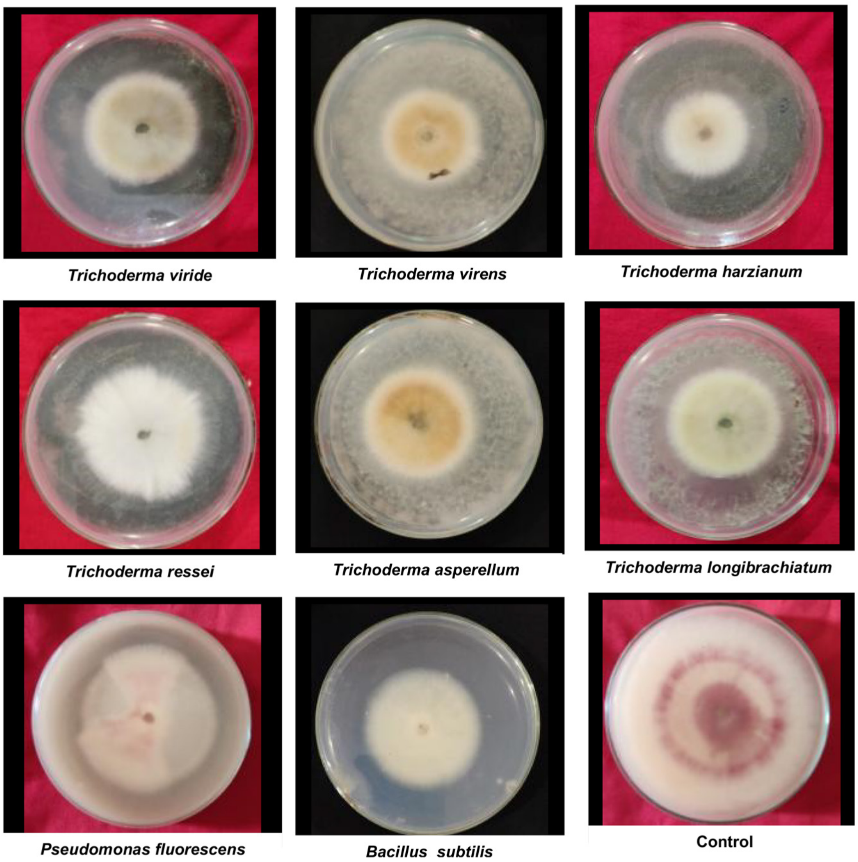

Fig. 2. Antagonistic efficacy of bioagents against *Fusarium oxysporum* f. sp. *pisi* by using volatile compound.

well observed in his *Trichoderma virid*e, *Bacillus subtilis* and *Pseudomonas fluorescens* bacteria. No sporulation was recorded with the other treatments.

Evaluation of antagonistic efficacy of volatile and non-volatile compounds of bioagents

Bioagents were also evaluated for their effect on test pathogen as they produce some volatile compounds during primary and secondary metabolism having tendency to vaporize which help in significantly reduce radial growth, percent growth inhibition and sporulation in *in vitro* and data on all presented in Table 1 and Fig. 2. Percent growth inhibition 86.80% was recorded highest in *Trichoderma harzianum* followed by *Trichoderma virens* (T_2- 71.93%) and *Trichoderma viride* (T_1- 63.45%). Lowest was recorded in *Trichoderma asperellum* (T_8- 53.03%) followed by *Trichoderma longibrachiatum* (T_6- 56.15%), and *Pseudomonas fluorescens* (T_4- 56.95%). Almost all treated plates results sporulation except *Trichoderma harzianum* there was no sporulation recorded. Excellent sporulation was recorded in control. *Trichoderma asperellum* and *Trichoderma viride* result good sporulation and poor sporulation was recorded in the remaining treatments.

Non-volatile compounds extracted from the liquid culture were assessed @ 5, 10 and 15% for their antifungal activity on radial growth, percent growth inhibition and sporulation of test pathogen and data presented in Table 2 and Fig. 3. Percent growth inhibition was recorded highest in *Trichoderma virens* (T_2- 82.01%), followed by *Trichoderma viride* (T_1- 64.74%), *Trichoderma harzianum* (T_3- 63.54%) and *Trichoderma ressei* (T_7-63.54%). Lowest inhibition was recorded in *Bacillus subtilis* (T_5-38.20%), followed by *Pseudomonas fluorescens* (40.76%), *Trich-*

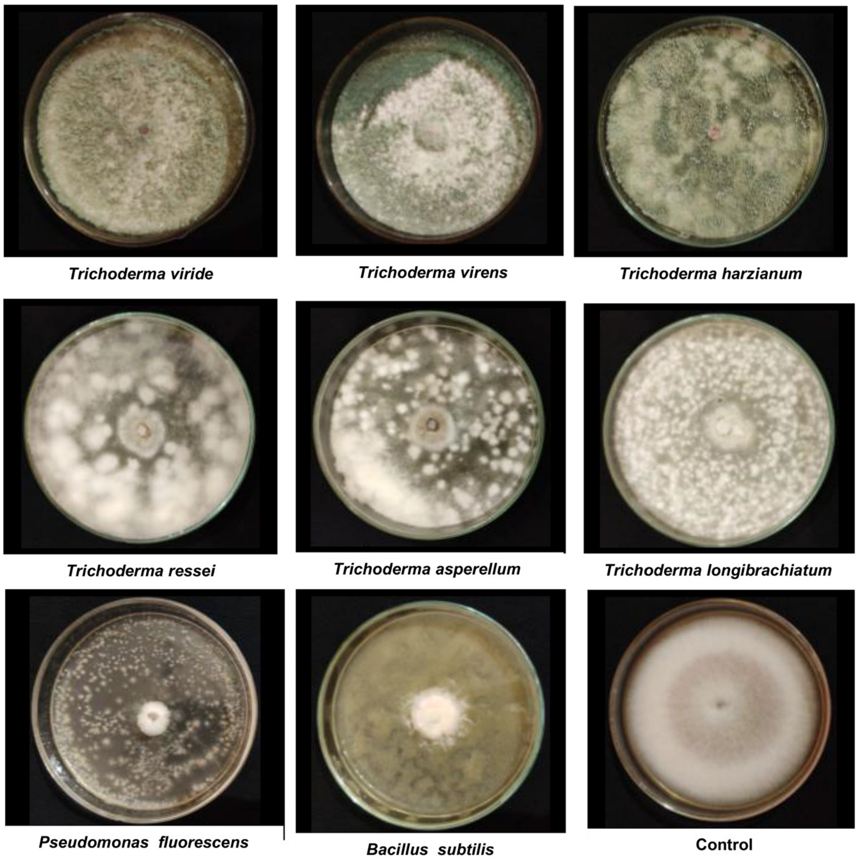

Fig. 3. Effect of non-volatile compounds (15%) of bioagents against *Fusarium oxysporum*.

oderma longibrachiatum (56.83%) and *Trichoderma asperellum* (62.47%). Sporulation was excellent in control plate. Good sporulation was recorded in *Pseudomonas fluorescens*. No sporulation was recorded in *Trichoderma virens*, *Trichoderma viride* and *Trichoderma harzianum* treated plate and remaining four show poor sporulation. At 10% growth inhibition was recorded highest in *Trichoderma virens* (T_2- 92.57%), followed by *Trichoderma viride* (T_1- 88.54%), *Trichoderma harzianum* (T_3- 85.15%) and *Trichoderma ressei* (T_7-73.90%). Lowest inhibition was recorded in *Bacillus subtilis* (T_5-51.10%), followed by *Pseudomonas fluorescens* (57.79%), *Trichoderma longibrachiatum* (70.17%) and *Trichoderma asperellum* (71.54%). Sporulation was excellent in control plate followed by *Bacillus subtilis*. Good sporulation was recorded in Pseudomonas fluorescens and *Trichoderma longibrachiatum*. No sporulation was recorded in *Trichoderma virens* treated plate and remaining four show poor sporulation ; and at 15% all tested bioagents were found effective and percent growth inhibition was recorded lowest in *Bacillus subtilis* (T_5-85.5%), followed by *Pseudomonas fluorescens* (87.00%), *Trichoderma asperellum* (89.0%) and *Trichoderma longibrachiatum* (93.6%). Sporulation was excellent in only control plate. No sporulation was recorded in any treatment except *Trichoderma asperellum* show poor sporulation.

DISCUSSION

As a potent and suitable biological control agent for managing soil-borne diseases, *Trichoderma* has grown in favor. *Trichoderma* species have shown potential against a number of significant soil-borne diseases, including *Pythium, Fusarium, Rhizoctonia*, *Sclerotium* and *Macrophomina* (Choudhary and Mohanka 2012, Kumar *et al*. 2012, Ommati and Zaker

Table 2. Effect of non-volatile compounds (5, 10 and 15%) of bioagents against *F. oxysporum* f. sp. *pisi* under *in vitro* of condition after 124 hrs of incubation.

	Treatment	5%			10%			15%		
		Radial growth of pathogen	% growth inhibition	Sporulation	Radial growth of pathogen	% growth inhibition	Sporulation	Radial growth of pathogen	% growth inhibition	Sporulation
T_1	*Trichoderma viride*	24.60	64.74	+	8.11	88.54	-	0.00	100.0	-
T_2	*Trichoderma virens*	12.55	82.01	-	5.26	92.57	-	0.00	100.0	-
T_3	*Trichoderma harzianum*	25.44	63.54	+	10.51	85.15	-	0.00	100.0	-
T_4	*Pseudomonas fluorescens*	41.33	40.76	++	29.87	57.79	++	10.12	87.0	-
T_5	*Bacillus subtilis*	43.12	38.20	+++	34.61	51.10	+	11.31	85.5	-
T_6	*Trichoderma longibrachiatum*	30.12	56.83	++	21.11	70.17	+	5.02	93.6	-
T_7	*Trichoderma ressei*	25.44	63.54	+	18.47	73.90	+	0.00	100.0	-
T_8	*Trichoderma asperellum*	26.19	62.47		20.14	71.54	+	8.61	89.0	+
T_9	Control	69.77	0.00		70.77	0.00	++++	78.01	0.0	++++
	SE (m)	**0.67**			**0.51**			**0.43**		
	CD (0.5)	**2.01**			**1.54**			**1.29**		

2012, Raut *et al.* 2014, Magar *et al.* 2020). Each of the investigated bioagents differentially restricted the pathogen's growth and increased the pathogen colony in the dual culture test as compared to the control. *Trichoderma virens* (81.97%) showed the greatest *Fusarium oxysporum* f. sp. *pisi* inhibition in solo culture, followed by *Trichoderma ressei* and *Trichoderma harzianum*. This result is in partial consonance with Kumar *et al.* (2009), Hamid *et al.* (2012), Kim and Knudsen 2013, Patel and Patel 2014, Kumar *et al.* (2016), Anuragi and Sharma (2016), Hassan *et al.* (2013).

By observing their radial growth and sporulation, the volatile compounds from the bioagents *Trichoderma viride*, *Trichoderma virens, Trichoderma harzianum, Trichoderma longibrachiatum, Trichoderma ressei, Trichoderma asperellum, Pseudomonas fluorescens* and *Bacillus subtilis* were assessed against *Fusarium oxysporum* f. sp. *pisi. Trichoderma harzianum* had the highest percent growth inhibition, followed by *Trichoderma virens* and *Trichoderma viride*. which conflicts with research by other researchers, including Singh *et al.* (2002), Kumar *et al.* (2009), Hamid *et al*. (2012), Kapoor *et al.* (2012), Kumar *et al.* (2014), Nagamani *et al*. (2016), Singh *et al.* (2016) and Cherkupally *et al.* (2017). Similar to this, the non-volatile components from bioagents were tested against *Fusarium oxysporum* f. sp. *pisi* at 5, 10, and 15% concentrations. The non-volatile substance from each individual *Trichoderma* treatment showed growth suppression of the test pathogen. Maximum *Fusarium oxysporum* f. sp. *pisi* growth and sporulation suppression was seen in *Trichoderma virens, Trichoderma harzianum* and *Trichoderma ressei* during single treatment. Many researchers, including Kumar and Dabbas(2016), Dubey *et al*. (2007) and Chaudhary *et al.* (2017), also obtained similar results. These outcomes provide strong support for the current conclusions.

Trichoderma harzianum SQR-T037, a new strain isolated by Raza *et al*. (2013), is tested *in vitro* for its ability to inhibit *Fusarium oxysporum* using both its volatile and non-volatile chemicals. To examine the antifungal effects of the volatile and nonvolatile metabolites generated by the *Trichoderma* T36 and T50 strains. A study was conducted by Raut and coworkers (2014) on *Fusarium graminearum*, *Rhizoctonia solani* and *Pythium ultimum* and discovered that the volatile component of *Trichoderma* T36 resulted in stronger inhibition than T 50 against all the pathogens. *Trichoderma virens, Trichoderma viride* and *Trichoderma harzianum* were the targets of Li *et al.* (2018)

research, which revealed that these bioagents had the potential to inhibit the growth of several *Fusarium oxysporum* strains. They also draw the conclusion that all species of *Trichoderma* can detect the volatiles produced by *Fusarium* spp. and can distinguish their presence by sensing them. *Trichoderma* then gets ready to combat pathogens by producing secondary metabolite compounds or volatile compounds. *Trichoderma asperellum* GDSF 1009 (CGMCC NO. 9512), *Trichoderma asperelloides* Z4-1 (CGMCC NO. 40245), *Trichoderma harzianum* 10569 (CGMCC NO. 40246), and *T. asperellum* 10264 (CGMCC NO. 22404) were combined to determine the best consortium for co-culture, the factors underlying the levels of *Fusarium oxysporum* antagonistic and growth-promoting activity in plants, as well as the enhancement of seed germination in monocultures of a single strain (Hao *et al.*2022).

CONCLUSION

Six spp. of *Trichoderma,* including *T. viride, T. virens, T. harzianum, Trichoderma longibrachiatum, T. ressei* and *T. asperellum* and two bacterial isolates *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for their volatile and non-volatile compounds, *in vitro* potential. All tested bioagents were effective against *Fusarium* in various degree. In dual culture *Trichoderma virens* found effective, in volatile compounds 86.80 % growth inhibition was recorded in *Trichoderma harzianum,* In non volatile compound, 100% growth inhibition was recorded in *Trichoderma viride, Trichoderma virens, Trichoderma harzianum* and in *Trichoderma ressei* at 15% concentration.

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