



Impact of *Trichoderma*-enriched Biofertilizer on the Growth and Yield of Mustard (*Brassica rapa* L.) and Tomato (*Solanum lycopersicon* Mill.)

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Abstract

Biofertilizer plays a significant role in crop cultivation with reduced the chemical fertilizer use. Three *Trichoderma*-enriched biofertilizers were evaluated in mustard and tomato cultivation at field condition. Sole application of biofertilizers didn't show remarkable contribution but all *Trichoderma*-enriched biofertilizers when supplemented with N fertilizer significantly boosted up the growth and yield of mustard and tomato. Application of 50% N fertilizer along with 50% *Trichoderma*-enriched biofertilizers augmented 108 and 203% yields over control both in mustard and tomato, respectively which were 81.90 and 61.82% in mustard and tomato at standard doses of Nitrogen, Phosphorus and Potassium fertilizers. The present results suggest that *Trichoderma*-enriched biofertilizer could save at least 50% N fertilizer uses for mustard and tomato and could reduce excessive uses of NPK for crop cultivation.

Keywords: *Trichoderma*, biofertilizer, crop growth, yield, biomass, tomato, mustard

1. Introduction

There is an agreement that among all the nutrients amendments applied to soil, N-fertilizer have by far the most important effects in terms of crop yield enhancement. There is increased emphasis on the environmental quality due to continuous use of chemical fertilizers. The integrated nutrient management i.e. combined use of chemical fertilizers with organic materials such as animal manures, crop residues, green manure and composts are alternatives and are characterized by reduced input of chemical fertilizers. Organic manures are well established to be useful in fertilization of plants due to their beneficial effect on the physical, chemical and

biological characteristics of the soil, which in turns, influence growth and increase plant production (Molla *et al.*, 2005; Meunchang *et al.*, 2006). In recent years, biofertilizers the products containing living cells of different types of microorganisms are also used in the integrated nutrient supply system. Biofertilizers can convert nutritionally important elements from unavailable to available form through biological processes leading to crop yields (Hegde *et al.*, 1999). Many researchers studied the role of organic manures, which was incorporated with biofertilizer as stimulating the plant growth and yield of vegetables (Shaheen *et al.*, 2007). Several studies have also shown that the use of compost in agriculture is beneficial to soil, crops

and environment (Rodd *et al.*, 2002; Rahman *et al.*, 2006). Moreover, compost acts as a long-term reserve and slow-release sources of major nutrient like N, P and K (Sullivan *et al.*, 2002).

Trichoderma harzianum, a filamentous fungus is used as a successful biological control agent to control different soil borne plant pathogens such as *Pythium* spp., *Rhizoctonia solani*, *Fusarium* spp., *Sclerotium rolfsii* etc (Harman *et al.*, 2004). It was demonstrated that *T. harzianum* induced defense responses and systemic resistance in addition to control of plant pathogens (Alfano *et al.*, 2007). Moreover, several species of *Trichoderma* promoted growth and development of seedlings of vegetable and non-vegetable crops (Bal and Altintas, 2006; Bal and Altintas, 2008). Furthermore, cucumber, bell pepper and strawberry yields were increased significantly by application of *Trichoderma* spp. (Elad *et al.*, 2006). Efficient use of *Trichoderma*-enriched biofertilizer may increase yield, reduce the uses of N fertilizers, reduce soil borne pathogens and improve soil health. The objectives of the present study were to investigate the impact of *Trichoderma*-enriched biofertilizer on yield and yield contributing characters and extent of reduction of N-fertilizers use alone or in combinations in tomato (*Lycopersicon esculentum* Mill.) and mustard (*Brassica rapa* L.) cultivation under field condition.

2. Materials and Methods

2.1. Experimental site, seed sowing and seedling transplantation

The experiments were carried out at the Agricultural Farm of the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh during winter season (Nov. 2008 to Feb 2009). The soil of the experimental site was analyzed for N based on Micro-Kjeldahl method (Bremner, 1960), for P by description of Olsen *et al.*, (1965) and for K based on procedure described by Jackson (1973). Mustard (variety- BARI mustard 16) seeds were sown in line on 1st December. Conversely, for

tomato (variety- Rattan) 1 month old, uniform and healthy single seedling was transplanted in each pit on 3rd December. Seedlings of tomato were grown in raised seed bed (5 g seeds were sown in seed bed of 3 m x 1 m) of sandy loam soil with addition of well decomposed compost (10 kg/bed). The unit plot size for both crops was 2.5 m x 2.0 m and spacing was 50 cm x 50 cm for tomato. For mustard, line spacing of 30 cm and plant to plant distance of 4-5 cm were maintained after thinning.

2.2. Source of *Trichoderma*-enriched biofertilizer (BioF)

Three biofertilizers enriched with *Trichoderma harzianum* were used in the present experiment. These were: (a) BioF/compost (*Trichoderma*-enriched organic compost i.e. household kitchen wastes were composted with *Trichoderma harzianum*); (b) BioF/pellets (*T. harzianum* coated wheat grains i.e. *T. harzianum* was allowed to grow over broken wheat grains); and (c) BioF/suspension (Broth of *T. harzianum* i.e. fungal spores-suspension were grown in liquid media, contained (g/l) KNO₃ 1.0, KH₂PO₄ 0.5, MgSO₄ 7H₂O 0.25, glucose 34, trace element FeCl₃ 0.5-1.0 and pH of 6.5 was maintained). Five pieces of 8 mm sized agar block of *Trichoderma* inocula were taken into 500 ml prior autoclaved (15 min at 121 °C) aforesaid media in 1000 ml Erlenmeyer flask from 4 days old culture grown in PDA (3.9% potato dextrose agar) media. Then the flasks were incubated at 150 rpm in orbital shaker for 7 days at room temperature. Afterward the fungal suspension was filtered by 12.5 cm Whatman # 1 filter paper. The obtained fungal spores culture was used as BioF/suspension (contained 3.5-6.15 × 10⁶ spores/ml approximately).

2.3. Fertilizer application and intercultural operations

Fertilizers were applied as per the soil analysis results for the respective experimental field. The doses of urea, triple super phosphate (TSP) and muriate of potash (MP) were 135, 54 and 5 g/plot respectively, for tomato. The equivalent

doses for mustard were 83, 36 and 5 g/plot. The total amount of TSP and MP and 1/3rd of urea were applied at final land preparation. The remaining urea was applied in two equal splits i.e. half at 30 days after transplanting/seed sowing and half at 60 days after sowing for mustard seeds and 75 days after transplanting for tomato seedlings. Biofertilizers such as BioF/compost, BioF/pellets and BioF/suspension were applied @ 618 g/plot, 5 g/plant and 5 ml/plant, respectively. The BioF/compost and BioF/pellets were applied to the soil immediately before sowing of mustard seeds and transplanting of the tomato seedlings. In case of mustard, BioF/suspension @ 25 ml was applied in each row at 2-3 days after seedling emergence with water by hand sprayer. On the other hand, BioF/pellets @ 25 g were dropped in each row before the sowing of seeds of mustard. In case of tomato, roots of the seedling were immersed in BioF/suspension and kept for approximately 30 min before transplanting. Five ml of BioF/suspension was applied in each pit immediately after the seedlings were transplanted. The intercultural operations viz. gap filling, thinning (only for mustard), weeding, staking, pruning, irrigation etc were done as needed.

2.4. Parameters considered for data collection

Four tomato and 10 mustard plants were randomly selected from each plot in such a way that the border effect was avoided. Data were collected for both tomato and mustard on plant height (cm), branches and leaves number per plant, root and shoot dry matter at 50% flowering stage (oven dried at 70°C for 3 days), clusters number per plant and number of flowers per cluster counted during full bloom. In addition, in case of tomato, data were also collected for number of fruits per plant, weight of individual fruit (g), and weight of fruits per plant (kg) at harvest. In case of mustard, additional data were collected on number of siliqua per plant, length of siliqua (cm), number of seeds per siliqua after harvest, seed yield per

plant (g) and yield per plot (g) after cleaning and drying.

2.5. Experimental design and statistical analysis

Experiments were conducted in a randomized complete block design (RCBD) with three replications and eight treatments. The assigned treatments were T₁ - control (without biofertilizer and chemical fertilizers), T₂ - NPK fertilizer, T₃ - BioF/compost (618 g/plot), T₄ - BioF/pellets (5 g/plant for tomato and 25 g/row for mustard), T₅ - BioF/suspension (5 ml/plant for tomato and 25 ml/row for mustard), T₆ - 50% N-fertilizer + 50% BioF/compost, T₇ - 50% N fertilizer + 50% BioF/pellets and T₈ - 50% N fertilizer + 50% BioF/suspension. Analysis of variance and comparison of means were calculated separately using a statistical package MSTAT-C (MSU, 1989). The treatment means were compared by the least significance difference (LSD) test. Based on LSD values different treatment means were separated from each other by different letters for easy interpretation.

3. Results and Discussion

3.1. Impact of Trichoderma-enriched biofertilizer (BioF) on vegetative growth

Trichoderma-enriched biofertilizer alone did not increase plant height, number of branches and number of leaves per plant in mustard compared to NPK fertilizer i.e. T₂ (Table 1). However application of *Trichoderma*-enriched biofertilizers along with N-fertilizer (except T₈) expedited those characters in mustard. The tallest plant (76.07 cm) was obtained in T₂ treated plots, which was significantly ($P \leq 0.01$) different from T₁, T₃, T₄, T₅ and T₈. The shortest plant (32.97 cm) was recorded in T₄, which was identical to that in T₅. As in case of plant height, almost similar observations were recorded for branch and leaf number per plant of mustard.

The impact scenario of *Trichoderma*-enriched biofertilizer in tomato was to some extent

different from the mustard (Table 1). The plant height of tomato was statistically identical in all treatments except T₅, which produced the lowest (46.50 cm) plant height. *Trichoderma*-enriched biofertilizer alone such as T₃ and T₄ treatments significantly increased the number of branches per plant in tomato but they were very similar to the treatments of T₂, T₆ and T₇. Alternatively, the number of leaves per plant was not increased by the sole application of *Trichoderma*-enriched biofertilizer such as the treatments T₃, T₄ and T₅. However, combined application (i.e. supplemented with N) of *Trichoderma*-enriched biofertilizer such as the treatments T₆, T₇ and T₈ increased the number of leaves per plant significantly ($P \leq 0.01$). The lowest number of leaves per plant (26.13) was observed in T₄, which was not different from T₁ and T₅.

The leaves number of both crops was significantly lower in sole application of biofertilizers (i.e. T₃, T₄ and T₅) and control. But when N fertilizer was supplemented with *Trichoderma*-enriched biofertilizers, it significantly increased all parameters of mustard but only leaf number of tomato. Moreover, the supplemented N fertilizer with *Trichoderma*-enriched biofertilizers did not create any impact on plant height and branch number of tomato, except in the treatment T₈. Perhaps, supplementation of N fertilizer enhanced the proliferation and growth of fungal microbes. The increased population of *Trichoderma* might play positive role to make available the plant nutrients in rhizosphere zone that increased growth and development of mustard and tomato. Plant growth stimulation by *Trichoderma* spp. and other microbes has been reported in several crops such as bean (Inbar et al., 1994), cucumber (Kleifeld and Chet, 1992), maize (Björkman et al., 1994), pea (Naseby et al., 2000) and tomato (Ozbay et al., 2004).

3.2. Response of *Trichoderma*-enriched biofertilizer on yield contributing characters

The sole application of *Trichoderma*-enriched biofertilizer (i.e. T₃, T₄ and T₅) in mustard

resulted in the lower number of flowers, number of siliqua and length of siliqua compared to T₂ (Table 2). In case of seed number, the sole application of *Trichoderma*-enriched biofertilizers provided almost similar result with T₂. Conversely, the numbers of flower and siliqua were significantly boosted up by supplementation of N fertilizer with *Trichoderma*-enriched biofertilizers, but the siliqua length and seed number did not respond in the same way.

In case of tomato, the supplemented N fertilizer with *Trichoderma*-enriched biofertilizers increased the fruit number and flower cluster (except T₆) significantly compared to sole application of biofertilizers, but the results were similar to T₂ (Table 2). However, the flower number per cluster did not respond significantly by combined treatments (i.e. T₆-T₈) compared to sole application of biofertilizer treatments.

The poorest performance of both crops (mustard and tomato) was observed in treatment T₅ but supplementation of N fertilizer to it i.e. treatment T₈ expedited the reproductive growth and yield of tomato significantly and not of mustard (Table 2). On the contrary, the highest performance was noted in most of the parameters at T₇ of both crops but poor performance was noticed in most cases at T₄ of mustard. The results showed that the supplemented N fertilizer with biofertilizers (i.e. 50% N + 50% BioF) significantly increased the number of flower per cluster, number of siliqua per plant of mustard and flower cluster and fruit number per plant of tomato, did not show any encouraging impact on siliqua length and seed number of mustard and flower number of tomato (Table 2). Perhaps, these characters of mustard and tomato are more genetically controlled i.e. poorly responsive. It has been reported that the strains of different *Trichoderma* species showed variable responses in cucumber, loofah and bitter gourd (Lo and Lin, 2002). Besides, it was reported that application of *Trichoderma* spp dramatically increased the number of fruits per plant in pepper and tomato grown in greenhouse than untreated control (Vinale et al., 2008).

Table 1. Effect of *Trichoderma*-enriched biofertilizer (BioF) alone or in combination with chemical fertilizers on the growth of mustard and tomato

Treatments	Mustard			Tomato		
	Plant height (cm)	Branch (no/plant)	Leaf (no/plant)	Plant height (cm)	Branch (no/plant)	Leaf (no/plant)
T ₁ (Control)	49.07 d	2.6 c	9.06 c	60.42 a	7.41 b	27.07 d
T ₂ (NPK fertilizers only)	76.07 a	5.9 a	25.20 a	64.58 a	7.83 ab	61.40 bc
T ₃ (BioF/compost)	60.33 bcd	3.2 bc	9.73 c	66.00 a	9.25 ab	55.60 c
T ₄ (BioF/pellets)	32.97 e	1.8 c	6.40 c	59.42 a	7.75 ab	26.13 d
T ₅ (BioF/suspension)	34.40 e	2.5 c	7.93 c	46.50 b	4.00 c	29.30 d
T ₆ (50% N + 50% BioF/compost)	73.03 ab	5.4 a	21.40 ab	66.17 a	8.25 ab	69.73 ab
T ₇ (50% N + 50% BioF/pellets)	64.33 abc	5.6 a	24.97 a	71.17 a	9.92 a	62.67 bc
T ₈ (50% N + 50% BioF/suspension)	52.90 cd	5.2 ab	13.67 bc	70.42 a	8.50 ab	73.77 a
CV (%)	10.61	20.46	24.56	8.14	12.26	7.76
LSD ($P \leq 0.01$)	14.02	1.97	8.66	12.24	2.3	9.39

In column common letters are not significantly differed at LSD ($P \leq 0.01$) level.

Table 2. Effect of *Trichoderma*-enriched biofertilizer (BioF) alone or in combination with chemical fertilizers on yield contributing characters of mustard and tomato

Treatments	Mustard				Tomato		
	Flower (per cluster)	Siliqua (no/plant)	Siliqua length (cm)	Seed (no/ siliqua)	Flower cluster (per plant)	Flower (no/cluster)	Fruit (no/plant)
T ₁ (Control)	3.08 c	31.20 bc	4.36 c	10.53 c	9.83 cd	5.45 bc	14.83 c
T ₂ (NPK fertilizers only)	8.08 a	55.53 ab	6.21 a	16.20 ab	19.22 ab	7.10 ab	36.83 a
T ₃ (BioF/compost)	5.25 bc	32.80 bc	5.82 ab	14.27 abc	21.67 a	7.00 ab	25.60 b
T ₄ (BioF/pellets)	3.08 c	29.20 bc	4.71 bc	15.67 abc	13.33 bc	6.17 abc	20.50 bc
T ₅ (BioF/suspension)	5.58 b	24.07 c	4.56 bc	12.40 bc	4.75 d	4.67 c	13.70 c
T ₆ (50% N + 50% BioF/compost)	5.83 ab	55.80 ab	5.54 abc	16.47 ab	16.42 abc	6.45 ab	35.97 a
T ₇ (50% N + 50% BioF/pellets)	6.08 ab	62.40 a	5.80 ab	19.07 a	21.00 a	7.75 a	41.03 a
T ₈ (50% N + 50% BioF/suspension)	6.25 ab	43.07 abc	5.06 abc	13.00 bc	18.73 ab	6.33 abc	39.43 a
CV (%)	18.85	29.62	10.80	14.70	17.97	11.67	13.04
LSD ($P \leq 0.01$)	2.43	29.50	1.35	5.15	6.69	1.76	8.86

In column common letters are not significantly differed at LSD ($P \leq 0.01$) level.

3.3. Impact of *Trichoderma*-enriched biofertilizers on yield of mustard and tomato

The highest seed yield (1.577 g) per plant of mustard was found in T₆, which was followed by T₂, T₃, T₅, T₇ and T₈ treatments (Table 3). Similarly, the seed yield per plot of mustard was also recorded the highest in T₆, which was similar to T₂, T₃, T₅ and T₇. The highest yield promotion (108.36 %) was attained in treatment T₆ and the second highest was 81.90 in T₂ treatment (Table 3). These results implied that the treatment T₆ could save at least 50% of N fertilizer.

Fruit yield of tomato per plant varied significantly among the treatments (Table 3). The maximum individual fruit weight was obtained in T₈, which did not differ significantly from treatment T₇ but was identical to T₂. The minimum individual fruit weight was recorded in control treatment (T₁). The highest fruit yield per plant (1.67 kg) was recorded in T₈, which was significantly ($P \leq 0.01$) different from with rest of the treatments. N fertilizer supplemented treatments T₆ and T₇ gave the second highest yield, which was significantly lower than the T₈ treatment, but significantly higher than the other treatments including T₂. The lowest fruit yield per plant (0.55 kg) was found in the control treatment. Yield increase of 203% was recorded in the treatment T₈, whereas 125 and 156% yield increase were observed in T₆ and T₇ treatments, respectively over control. The yield increase of 61.82% was observed in T₂ over control. However, 56, 31 and 9% were observed in sole biofertilizers i.e. T₃, T₄ and T₅ treatments, respectively over the control. The yields were significantly influenced by the combined treatments i.e. T₆T₈ by supplementation of N fertilizer to the biofertilizers. These results implied that yield of tomato was significantly increased when *Trichoderma*-enriched biofertilizers were combined with chemical N fertilizer (Table 3). The combined treatment gave significantly higher yield of tomato than the treatment T₂ i.e. standard dose of NPK fertilizer.

Generally, the NO₃⁻ form of N fertilizer couldn't retain long time to the rhizosphere zone of plants and therefore, split application of N fertilizer was suggested at different stages of crop growth. Conversely, the organic amendments and microbes played a role to slow by release the plant nutrients. The microbial population in soil releases some exudates (organic substances), increases nutrient uptake through enhanced root growth or promotes availability of necessary nutrients and solubilize a number of poorly soluble nutrients, such as Mn⁴⁺, Fe³⁺ and Cu²⁺ etc (Kleifeld and Chet, 1992; Altomare *et al.*, 1999). Therefore, in present study the combined treatments enhanced efficient utilization of nutrients than the others which expedited crop growth and yield.

3.4. Impact on dry matter production

Influence of different forms of *Trichoderma*-enriched biofertilizers alone or in combination with N fertilizer on dry matter production of mustard was also noticed in the present study (Table 4). The highest root (0.66 g/plant) and shoot (6.83 g/plant) dry matter weight of mustard was found in T₆ treatment. The lowest root dry matter (0.11 g) was recorded in T₁, which was followed by T₄ treatment. Shoot dry weight followed the similar trend as root dry weight. There clearly illustrated that dry matter production was boosted up by the supplementation of partial N fertilizer to each biofertilizer application. The scenario of root and shoot dry weight of tomato also followed the similar profile as mustard (Table 4). Similar trend of results was noticed in corn shoot dry weight using *T. harzianum* (Björkman *et al.*, 1994). Increased root dry weight by application of *Trichoderma*-enriched biofertilizer might be one of the important factors for enhancing growth and yield of mustard and tomato. Furthermore, the increased root biomass could increase the chance for acquisition of nutrients by exploitation of more volume of soils.

Table 3. Effect of *Trichoderma*-enriched biofertilizer (BioF) alone or in combination with chemical fertilizers on yield of mustard and tomato

Treatments	Mustard			Tomato		
	Seed yield (g/plant)	Yield (g/plot)	Percent yield increased	Individual fruit wt.(g)	Fruit yield (kg/plant)	Percent yield increased
T ₁ (Control)	0.529 b	69.16 cd	-	62.27 d	0.55 d	-
T ₂ (NPK fertilizers only)	1.303 ab	125.80 ab	81.90	82.00 ab	0.89 c	61.82
T ₃ (BioF/compost)	0.913 ab	99.05 abc	43.21	71.87 c	0.86 c	56.36
T ₄ (BioF/pellets)	0.533 b	71.36 cd	3.19	71.03 c	0.72 cd	30.90
T ₅ (BioF/suspension)	1.213 ab	101.54 abc	46.82	72.50 c	0.60 d	9.09
T ₆ (50% N + 50% BioF/compost)	1.577 a	144.1 a	108.36	74.43 bc	1.24 b	125.45
T ₇ (50% N + 50% BioF/pellets)	1.280 ab	101.1 abc	46.18	86.57 a	1.41 b	156.36
T ₈ (50% N + 50% BioF/suspension)	0.773 ab	79.27 bcd	14.18	87.17 a	1.67 a	203.63
CV (%)	33.98	24.04	-	4.49	9.0	-
LSD ($P \leq 0.01$)	0.88	51.16	-	8.14	0.21	-

In column common letters are not significantly differed at LSD ($P \leq 0.01$) level.

Table 4. Dry matter production of mustard and tomato as influenced by *Trichoderma*-enriched biofertilizer (BioF) alone or in combination with chemical fertilizers

Treatments	Mustard		Tomato	
	Root weight/plant (g)	Shoot weight/plant (g)	Root weight/plant (g)	Shoot weight/plant (g)
T ₁ (Control)	0.11 e	2.09 e	0.11 g	2.07 d
T ₂ (NPK fertilizers only)	0.34 c	5.02 c	0.38 d	4.36 c
T ₃ (BioF/compost)	0.27 cd	3.68 d	0.32 de	3.96 c
T ₄ (BioF/pellets)	0.14 e	2.45 e	0.28 ef	2.40 d
T ₅ (BioF/suspension)	0.19 de	2.05 e	0.21 f	2.54 d
T ₆ (50% N + 50% BioF/compost)	0.66 a	6.83 a	0.69 a	6.57 a
T ₇ (50% N + 50% BioF/pellets)	0.58 ab	5.70 b	0.59 b	5.42 b
T ₈ (50% N + 50% BioF/suspension)	0.54 b	5.26 bc	0.48 c	5.08 b
CV (%)	11.78	5.86	7.86	5.37
LSD ($P \leq 0.01$)	0.106	0.579	0.075	0.517

In column common letters are not significantly differed at LSD ($P \leq 0.01$) level.

In general, the application of biofertilizers in different form enhanced dry matter production of mustard and tomato. The dry matter production was boosted up while biofertilizers were supplemented with N fertilizer. The growth, maintaining population and adaptation of microbes in rhizosphere zones are determined to some extent by the root exudations. The impact of compost on superior plant growth was most likely due to increase in N availability as well as its slow and steady release in growing media (Erhart *et al.*, 2005). Moreover, the compost treated plants might obtain some additional plant nutrients for growth and development (Meunchang *et al.*, 2006).

4. Conclusions

In the present study, it was clearly observed that the *Trichoderma*-enriched biofertilizer had positive impact on growth and yield of mustard and tomato. The impact of *Trichoderma*-enriched biofertilizer accelerated when it was supplemented with N fertilizer application. Superior and significant growth, yield and dry matter production were increased by supplementation of N fertilizer with *Trichoderma*-enriched biofertilizer. Maximum of 108.36% yield increase of mustard over control was recorded in T₆ i.e. combined treatment, which was 92% of control. Similarly, the maximum 203.63% yield increase over control of tomato was noticed in T₈, which was 61.82% in T₂. The responses of both crops to biofertilizers were not alike. It may be concluded that application of *Trichoderma*-enriched biofertilizer along with N fertilizer could save at least 50% N fertilizer giving higher yields in tomato and mustard compared to T₂ (NPK treatment). Undoubtedly, there is a prospective and potential of *Trichoderma* contained biofertilizer in crop cultivation to achieve attractive yield and reducing N-fertilizer application.

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