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# Evaluation Effects of Chemical, Organic and Biologic Fertilizers on Chamomile (*Matricaria chamomile* L.) Yield

## Fatemeh Jahani<sup>1</sup>, Abbas Maleki<sup>\*2</sup>, Alireza Pazoki<sup>3</sup>

1- Young Researchers and Elite Club, Yadegar-e-Imam Khomeini Shahr-e-Rey Branch, Islamic Azad University, Tehran, Iran.

2- Department of Agronomy and Plant Breeding, Ilam Branch, Islamic Azad University, Ilam. Iran.

3- Department of Agronomy and Plant Breeding, Yadegar-e-Imam Khomeini Shahre-Rey Branch, Islamic Azad University, Tehran, Iran.

RESEARCH ARTICLE

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## ABSTRACT

Although herbs have been important and attractive since long time ago, they have gained such popularity due to the adverse side effects of chemical medicines that nowadays their cultivation has become among significant agricultural programs of most developed countries. In order to study the effects of chemical, organic, and biological fertilizers on Medicinal Chamomile yield and its components, a split plot experiment laid out in a randomized complete blocks design was conducted with three replications. The experimental fertilizing factors were applied as follows: chemical N as the main factor at four different levels (0, 50, 100, and 200 kg.ha<sup>-1</sup>), manure as the secondary factor at two different levels (0 and 30 kg.ha<sup>-1</sup>), and Nitroxin, an organic fertilizer, as the subordinate factor at two levels (not inoculating versus inoculating Chamomile seeds). Results revealed that chemical N and organic fertilizers significantly affected plant height, number of branches per plant, number of seeds per capitols, seed yield, 1000 seeds yield, and Nitrogen use efficiency, but there were no significant effects on root length and number of capitols per plant. The number of capitols per plant was not affected by any of the studied factors, but root length was only affected by biological fertilizer. Compared to the control, seed yield was increased 53%, 53%, and 59% respectively. Among these three experimented fertilizers, the biological Nitroxin was the most influential one on the above mentioned traits.

Keywords: Nitrogen use efficiency, Oil yield, Urea.

#### INTRODUCTION

Herbs have been traditionally prescribed as a disinfectant since years before Christ and their application in curing diseases has coincided mankind life (Dermadrosian, 2001). Chamomile is a plant derived from Asteraceae tribe and its subordinate Radiae (Sato et al., 2006). This plant contains 0.24- 1.9% volatile oil and more than 120 identified compounds including *a*-Bisabolol and Chamazulene (Baghalian et al., 2008). Since Chamomile contains Flavonoids, its different therapeutic effects includes anti-inflammatory, antiemetic, antibacterial and antispasmoic ones (McKay and Blumberg, 2006). The quality of plant medicinal compounds depends on plant physiological potential and its growth environmental factors as well as its nutrition (Hecl and Sustrikova, 2006). Biological fertilizers are also considered as microbial inoculants capable of transforming immobilized soil nutrients to their available forms, through biologic processes, and providing the crops with them (Wu et al., 2005; Fathi et al., 2013). Considering Nitrate leaching in humid regions and the resulted high Nitrate concentration of ground waters, ammonia sublimation and denitrification under submerged conditions (Fageria and Baligar, 2005). the application of N fixing growth stimulating bacteria during plant growth seems to be useful, since they fix N and make it available for the plants (Barea et al., 2002; Zaied et al., 2003). More ever, organic matters improve soil physical, chemical, and biological properties, therefore mineral material would become water soluble and exchangeable in soil or become a part of organic matters released gradually and absorbed by plants, all these would result in minimized soil erosion and nutrients leaching (Manna et al., 2007). This study was conducted in order to develop sustainable agriculture during the transition from traditional to modern agriculture. On the other hand, sustainable agriculture strategy insists on high yield through executing an agricultural system with sufficient input. In this regard, integrating chemical, biological and organic fertilizers as a solution for alternative agricultural approach seems to be a suitable and useful mechanism to maintain the acceptable level of yield and crop production.

#### MATERIALS AND METHODS

## **Geographical Location of Experiment**

This experiment was conducted in Sarableh Agricultural center field during the spring of 2014. It is located in the southern slopes of Kabirkooh in Ilam province, in 32° 33' 58 N and 47° 07 09' E. According to Copen category caste, it benefits a moderate mountain climate with an annual precipitation rages from 400-600 mm and an absolute temperature varies from -6.5-39 °C. Its altitude is 1050 meters.

## **Experimental Design and Treatments**

A split plot experiment laid out in a randomized complete blocks design was conducted with three replications. The experimental N fertilizing factors were applied as follows: chemical N as the main factor at four levels (0, 50, 100, and 200 Kg.ha<sup>-1</sup>), livestock manure at two levels (0 and 30 Kg.ha<sup>-1</sup>), and Nitroxin, an organic fertilizer, at two levels (not inoculating versus inoculating Chamomile seeds). Each experimental unit consisted of 8 furrows. The furrows were 4 meters long and 30 cm far from each other. The distance of the plants was considered to be 10 cm. A furrow was left uncultivated between every two plots and the distance between each replication was determined to be 3 meters. Seeds were cultivated on cultivation

line and 2-3 cm below soil surface. Soil preparation operate (Including plowing, disking and troweling) were favorably carried out before cultivation step.

#### Soil Analysis

In order to determine soil properties before conducting the experiment, sampling was carried out from 0-30 cm soil depth and its properties were investigated. According to results of the analysis, the soil had a clay loam texture. Results of soil test mentioned in table 1.

Table1.	Soil	physical	and	chemical	l proper-
	•	0.1	•	1 0 1	1

ties of the experimental field						
Soil depth	0-30 cm					
Bulk Density (gr.cm <sup>-2</sup> )	1.59					
EC (ds.m <sup>-1</sup> )	3.1					
Clay (%)	26					
Sand (%)	39					
Silt (%)	35					
Soil acidity (pH)	7.3					
N (%)	0.06					
Organic Carbon (%)	0.75					

#### **Biological Fertilizer**

Nitroxin was selected as the biological fertilizer, since it contains most effective N fixing, namely Azotobacter and Azospirllum, and phosphate solubilizer bacteria, Pseudomonas. These bacteria contribute to the absorption of macronutrients and micronutrients by the plant. Azotobacter and Azospirllum are also able to biologically fix N (Sharifi and Haghnia, 2007). Before cultivation step and according to above mentioned amounts, Different levels of N and organic fertilizer were applied during soil preparation step. Urea was used as N fertilizing treatment. A suitable amount of inoculant (1 lit per 30 Kg of seeds) was gradually sprayed on seeds, then seeds were continuously mixed to be inoculated and were left under shadow to be completely dry. After this step, they were ready to cultivated (Fathi et al., 2013; Akbari et al., 2009).

#### Yield and Seed Yield Components

During physiological maturation stage, 6 plants were picked up from each plot and some of their properties including root length, plant height, number of secondary branches per plant, number of capitols per plant, and number of seeds per capitol were measured. After physiologic maturation stage and when plants became completely dry, the final harvest was carried out neglecting the margins (that is, a 2 m<sup>2</sup> area), and other variables such as seed yield and 1000 seeds weight were computed and determined.

#### Nitrogen use efficiency

Nitrogen use efficiency was computed based on seed yield and through diving it on the available N of each treatment (fertilizing compound). Regarding Urea fertilizer, its available N was determined to be 46% and for the manure, it was measured to be 0.2% (the sum of available N in Urea and manure).

**Equ.1.** Seed Nitrogen use efficiency = Seed yield per area  $(m^2)/Available N$  of fertilizing compound per area  $(m^2)$ 

#### Statistical Analysis

Data were variance analyzed by SAS software, and means were compared via LSD test at 5% probability level.

#### **RESULTS AND DISCUSSION**

Results of the current study indicated that among the applied treatments, only Nitroxin significantly affected root length and other treatments had no significant effects on this trait (Table 2). The longest root was obtained from biological fertilizer inoculated seeds treatment with 19.4 cm length and the shortest one was from the not inoculated seeds treatment with 15.6 cm length (Table 3).

S.O.V	df	Root Length	Height	Number of Branches per Plan	Number of Seeds per Capitol	Number of Capitol Plant	1000 Seeds Weight	Seed yield	Nitrogen use efficiency	Essential oil yield
Repetition	2	16.26 <sup>ns</sup>	101.64**	617.01**	41.86 <sup>ns</sup>	485.50 <sup>ns</sup>	0.0013*	6865 <sup>ns</sup>	2.203 <sup>ns</sup>	489040 <sup>ns</sup>
Nitrogen (N)	3	1.38 <sup>ns</sup>	141.95**	1123.7**	62.91*	$16.72^{ns}$	0.0036**	13123*	15.291**	636330 <sup>ns</sup>
Main Error	6	0.60	5.31	8.29	11.48	109.56	0.002	1922	0.400	344809
Manure	1	8.90 <sup>ns</sup>	675**	4831.6**	410.67**	345.08 <sup>ns</sup>	0.0088**	75167**	20.391**	7580965**
$N \times Manure$	3	6.06 <sup>ns</sup>	8.50 <sup>ns</sup>	$119.52^{*}$	8.09 <sup>ns</sup>	$4.52^{ns}$	$0.0002^{ns}$	1170 <sup>ns</sup>	4.318*	140051 <sup>ns</sup>
Secondary Error	8	4.17	12.24	21.08	4.59	74.24	0.0004	2618	0.558	377172
Biological Fertilizer	1	170.65**	1759.34**	14227.2**	517.45**	309.07 <sup>ns</sup>	0.0125**	88966**	21.048**	7069035**
N × Biological Fertilizer	3	0.29 <sup>ns</sup>	19.31 <sup>ns</sup>	169.36*	15.58 <sup>ns</sup>	0.62 <sup>ns</sup>	0.0009**	3205 <sup>ns</sup>	3.152**	397517 <sup>ns</sup>
Manure × Biologi- cal Fertilizer	1	4.76 <sup>ns</sup>	33.33 <sup>ns</sup>	89.81 <sup>ns</sup>	92.96*	3.00 <sup>ns</sup>	0.0000 <sup>ns</sup>	7603*	1.763 <sup>ns</sup>	1361758*
N × Manure × Bio- logical Fertilizer	3	10.01 <sup>ns</sup>	36.43 <sup>ns</sup>	479.3**	39.39 <sup>ns</sup>	11.36 <sup>ns</sup>	0.0007**	5403*	0.065 <sup>ns</sup>	586110 <sup>ns</sup>
Total Error	16	3.77	12.64	43.78	15.37	68.99	0.0001	1217	0.389	187219
CV (%)		11.1	7.8	12.2	8.7	7.9	11.4	8.9	16.6	15.5

Table 2. Result of analysis of variance of studied traits

<sup>ns, \* and \*\*</sup>: no significant, significant at 5% and 1% of probability level, respectively.

Researchers suggested that phosphate solubilized bacteria, which are capable of producing AAC deaminase, boost root growth and increase its length. They concluded that in addition to phosphate solubilizing trait, other factors have stimulated plant growth. The production of organic acids by the micro organisms are among these factors. The produced organic acids would acidify microbial cell and its environment, so due to proton substitution, phosphorus is released instead of Calcium (Zabihi *et al.*, 2009).

#### **Plant Height**

Results of the study showed that the effect of N, manure, and biological fertilizer on plant height was significant (Table 2). The tallest plant (49.3 cm) was obtained from 200 Kg.ha<sup>-1</sup> N application treatment. On the other hand, the shortest plant (41 cm) was produced in the control (not applying fertilizer). Additionally, all treatments had statistically different effects on plant height (Table 3). Rahmani et al., (2007) also indicated that N treatment had a significant effect on the height of Marigold and the tallest plant, with a height equals 31.8 cm, was observed in 90 Kg.ha<sup>-1</sup> N application treatment. However, their results are not consistent with results of this study. Habibi and Imanikhah (2006) reported that N did not significantly affect German Chamomile height. By applying manure, the height of plant which was 41.6 cm, increased and became 49.1 cm. The shortest plant (41.6 cm) was picked up from not applying manure treatment (Table 3).

Treatments		Root length (cm)	Plant height (cm)	Number of seeds per capitol	Nitrogen use efficiency	Essential oil yield (g.ha <sup>-1</sup> )	
	0	17.8* <sup>a</sup>	41 <sup>d</sup>	29.6 <sup>d</sup>	4.18 <sup>c</sup>	933.1 <sup>a</sup>	
Nitrogen	50	17.1 <sup>a</sup>	$44.8^{\circ}$	31°	2.77 <sup>b</sup>	167.8 <sup>a</sup>	
(Kg.ha <sup>-1</sup> )	100	17.3 <sup>a</sup>	46.4 <sup>b</sup>	32.5 <sup>b</sup>	1.95 <sup>a</sup>	1274.5 <sup>a</sup>	
	200	$17.8^{a}$	49.3 <sup>a</sup>	35 <sup>a</sup>	2.77 <sup>b</sup>	1486.8 <sup>a</sup>	
Manure	Not Applying	17.1 <sup>a</sup>	41.6 <sup>b</sup>	29.1 <sup>b</sup>	4.18 <sup>a</sup>	8181.1 <sup>b</sup>	
	Applying	17.9 <sup>a</sup>	49.1 <sup>a</sup>	34.9 <sup>a</sup>	1.95 <sup>a</sup>	1612.9 <sup>a</sup>	
Biologic Fertilizer	Not Inoculating	15.6 <sup>b</sup>	39.3 <sup>b</sup>	28.7 <sup>b</sup>	4.18 <sup>b</sup>	831.8 <sup>b</sup>	
	Inoculating	19.4 <sup>a</sup>	51.4 <sup>a</sup>	35.3 <sup>a</sup>	4.18 <sup>c</sup>	1599.3 <sup>a</sup>	

**Table 3.** The means comparison effect of treatments on studied traits

\*Similar letters in each column show non-significant difference at 5% probability level in Duncan test.

Probably the increase in plant height at the end of growth season is due to the gradual release of manure nutrients and their subsequent absorption and accumulation in the plant stem. More ever, in terms of seed inoculation, the height was 39.3 cm in not inoculated treatment and in the inoculated seeds treatment the tallest plant had 51.4 cm height (Table 3). Shaalan (2005), also concluded that inoculating Nigella Sativa with the Azospirllum, Azotobacter and Pseudomonas improved plant growth properties (Including plant height). Perhaps the main reason beyond this improvement was the increase in nutrients absorption by the plant.

## Number of Branches per Plant

All three fertilizers had significant effects on the number of branches per plant (Table 2). The findings of this study revealed that the triple effect on this trait was significant too (Table 2). The highest number of branches per plant was obtained from inoculated seeds and 50 Kg.ha<sup>-1</sup> Urea application treatments which reached 106.4, but the lowest number (34.8) was observed in not inoculated seeds and not applying Urea and manure treatments (Table 4). Probably, the low number obtained in the control is due to nutrient deficiency which also confirms that applying N. organic, and biological fertilizers associated with supplying required nutrients and high available moisture lead to high number of secondary branches per each Chamomile plant. Molaphilabi et al., (2009) in their study on Nigella Sativa demonstrated that nitrogen increases the number of flowering branches per plant. Jahan and Koochaki (2004), reported that applying 30 t.ha<sup>-1</sup> of manure increased the number of secondary branches of Chamomile. Bahamin (2011) believed that the high number of branches in the Nitroxin inoculated treatment was due to higher vegetative growth.

#### Number of Seeds per capitol

Results of this study indicated that N, manure, and Nitroxin significantly affected the number of seeds per capitol (Table 2). In this research, 200 Kg.ha<sup>-1</sup> N application treatment produced the highest number of seeds per capitol (35) and the lowest number was obtained from the control (not applying N) which was 29.6. Different levels of N fertilizer had significantly different effects on this trait (Table 3). Findings have suggested that the interaction of biological fertilizer and manure significantly affected this number. The highest number of seeds per capitol (39.6) was obtained from seed inoculation and manure application treatment. In contrast, the lowest number (27.2) was produced in not inoculated and not manure applied treatment (Table 3).

Biological Fertilizer	Manure Urea (Kg.ha <sup>-1</sup> ) (Kg.ha <sup>-1</sup> )		Seed yield (Kg.ha <sup>-1</sup> )	Number of branches per plant	1000 seeds weight (gr)	
	0	0	110.4 <sup>*gh</sup>	53 <sup>gh</sup>	$0.042^{fg}$	
	0	50	163.7 <sup>e-h</sup>	66.5 <sup>ef</sup>	$0.050^{d}$	
	0	100	204.5 <sup>bc</sup>	81 <sup>cd</sup>	0.056 <sup>bcd</sup>	
	0	200	239.8 bcd	91.2 <sup>bc</sup>	$0.059^{ab}$	
Inoculation						
	30	0	263.2 <sup>abc</sup>	78.2 <sup>bcd</sup>	$0.058^{ab}$	
	30	50	315.3 <sup>a</sup>	106.4 <sup>a</sup>	0.063 <sup>a</sup>	
	30	100	287.6 <sup>ab</sup>	99.3 <sup>ab</sup>	0.061 <sup>ab</sup>	
	30	200	268.5 abc	90 <sup>bc</sup>	$0.060^{ab}$	
	0	0	106.5 <sup>gh</sup>	34.8 <sup>i</sup>	0.037 <sup>g</sup>	
	0	50	95.2 <sup>h</sup>	39.3 <sup>i</sup>	$0.038^{\mathrm{fg}}$	
	0	100	103.5 <sup>gh</sup>	37.7 <sup>i</sup>	$0.039^{\mathrm{fg}}$	
Not	0	200	168.5 <sup>efg</sup>	53.1 <sup>gh</sup>	0.052 <sup>cd</sup>	
Inoculation	30	0	123.2 <sup>fgh</sup>	34.4 <sup>hi</sup>	$0.043^{fg}$	
	30	50	143.2 <sup>e-h</sup>	52.5 <sup>gh</sup>	0.045 <sup>ef</sup>	
	30	100	178.7 def	61.2 <sup>fg</sup>	0.051 <sup>d</sup>	
	30	200	244.5 <sup>bc</sup>	77.2 <sup>de</sup>	$0.062^{ab}$	

Table 4. Mean comparison interaction effects of treatments on studied traits

\*Similar letters in each column show non-significant difference at 5% level in Duncan's multiple rang test.

Perhaps, through N biological fixation by growth stimulating bacteria, more N is transferred toward capitols which leads to higher cell reproduction rate and higher number of florets, hence more seeds are produced in each capitol. Other researcher have also reported the influence of nitrogen fertilizers on the higher number of seeds per plant for Marigold (Naghdi Badi *et al.*, 2002), Fleawort (Yadav *et al.*, 2002), and Chamomile (Fallahi *et al.*, 2009).

## Number of Capitols per Plant

Results of the study indicated that no treatment had a significant effect on the number of capitols per plant (Table 2). Probably, this trait is mainly controlled by genetic factors, thus the studied treatments did not significantly affect it. Rahmani (2007) also reported that manure and N fertilizer did not significantly affect the number of capitols per plant. Akbarinia *et al.*, (2005), stated that applying N did not significantly affect the number of Cumin reproductive organs.

#### Seed Yield

According to the results, all treatments significantly affected seed yield (Table 2). Compared to the control, the application of N (200 Kg.ha<sup>-1</sup> N), organic, and biologic fertilizers increased seed yield (53, 53, and 59% respectively). The triple effect of all treatments on this trait was significant. The highest seed yield (315.3 Kg) was obtained from 50 Kg N + manure + biologic fertilizer treatment (Table 4). The lowest seed yield (95.2 Kg) was observed in not applying any of the fertilizers treatment. Normally, inoculating the seeds and applying manure would decrease N fertilizers demands. Based on these results, it can be said that nitrogen is an essential element for producing high yields of herbs, including

Chamomile, but its excessive application should be prevented, since this would decrease the yield. More ever, applying a combination of N, organic, and biologic fertilizers can decrease the need for chemical N fertilizers. Therefore, it can be concluded that, compared to chemical fertilizers, the application of biological fertilizers, whether alone or in combination with other fertilizers, would increase seed yield of most herbs. Ahmad et al., (2010) reported that higher seed yield due to the application of N fixing biologic fertilizers may be resulted from the increase in metabolic activities of these fertilizers, which in turn accelerates pure photosynthesis rate, and the production of growth stimulating hormones by the bacteria and eventually increases the vield.

#### **1000 Seeds Yield**

Results of the current study revealed that N fertilizer, manure, and Nitroxin, as well as their triple interaction significantly affected 1000 seeds yield (Table 2). Findings indicated that the triple interaction of all treatments significantly affected this trait. The heaviest 1000 seeds yield (0.063 gr) came from 50 Kg N + manure + biologic fertilizer treatment, which was not significantly different from 200 Kg N+ manure + biologic fertilizer treatment. The lightest 1000 seeds yield (0.037 g) was obtained from not applying any fertilizer treatment which showed a significant difference with most of the treatments (Table 4). Other researchers have also reported the positive effect of combinatory application of these three fertilizers (Yadav et al., 2008; Pouryosef et al., 2007; Fathi et al., 2013: Singh et al., 1988). It seems that combinatory application of these fertilizers along with gradual nutrients release, which is consistent with plant growth cycle, the rise

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in available water, and the improvement of soil physical structure would accelerate and prolong plants photosynthesis rate which will finally increase seed weight.

## Nitrogen use efficiency

Results of this study indicated that all the treatments have significant effects on Nitrogen use efficiency (Table 2). These findings also suggested a significant effect of combinatory application of N and biological fertilizers on this variable. The lowest Nitrogen use efficiency (1.7%) was observed in 200 Kg.ha<sup>-1</sup> N application and not inoculated treatment. On the other hand, the highest Nitrogen use efficiency (5.4%) belonged to 50 Kg.ha<sup>-1</sup> N application and inoculated treatment. Also, all N fertilizer levels showed a higher efficiency when were applied on inoculated treatments, compared to not inoculated ones (Table 3). Bahamin (2011), in one of his researches reported that biological fertilizers significantly affected Nitrogen use efficiency of seeds. He also suggested that biological fertilizers don't have N by themselves, but via their N fixing bacteria, they able to improve Nitrogen use efficiency. More ever, the interactive effect of N fertilizer and manure was significant on Nitrogen use efficiency. The lowest Nitrogen use efficiency (1.7%) was observed in 200 kg.ha<sup>-1</sup> N fertilizer and not manure application treatment. The highest Nitrogen use efficiency (5.6%) was obtained from 50 Kg.ha<sup>-1</sup> N and not manure applied treatment. Also, all N fertilizer levels showed a higher efficiency when were applied in the absence of manure treatments, compared to applied manure ones (Table 3). It seems that the highest fertilizer efficiency is obtained in its first consumed units, and as its amount gradually increases, plant nutrients deficiency is made up for, but applying

more and more fertilizers would decrease its efficiency. In fact, consumption efficiency is a suitable index to assess the efficiency of fertilizers or nutrients in improving yield and yield components. These results were consistent with those of Dastborhan *et al.* (2011) on Chamomile and Abaszadeh *et al.*, (2007) on Lemon Balm.

## Essential oil yield

Results of the study showed that manure, biological fertilizer, and their interaction significantly affected Essential oil yield, but N fertilizer had no significant effect on this variable (Table 2). By applying manure, Essential oil yield reached to 1612.9 g.ha<sup>-1</sup> which, compared to not applying manure, portended a 97% increase. In this research, inoculating seeds with biological fertilizer raised this variable from 831.8 g/ha in not inoculated treatment to 1599.3 g.ha<sup>-1</sup> (Table 3). Results revealed that the interactive effect of manure and biological fertilizer on Essential oil yield was more efficient than the effect of their segregated application. The highest Essential oil yield (2165.1 g.ha<sup>-1</sup>) was observed in manure applied inoculated treatment which, compared to the control, showed a 259% increase. In a research conducted by some researchers to investigate the effect of chemical and manure application, as well as their interaction on Carum Copticum vield. Essential oil yield, and essence main compounds, it was reported that chemical fertilizers increased seed yield, but had no significant effect on seed Essential oil yield (Letchamo and Vomel, 1993).

#### CONCLUSION

Chemical N and the organic fertilizers significantly affected plant height, number of branches per plant, number of seeds per capitols, seed yield, 1000 seeds yield, and Nitrogen use efficiency, but there were no significant effects on root length and number of capitols per plant. The number of capitols per plant was not affected by any of the studied factors, but root length was only affected by biological fertilizer. Compared to the control, seed yield was increased 53%, 53%, and 59% respectively. Among these three experimented fertilizers, the biological Nitroxin was the most influential one on the above mentioned traits.

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