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Effect of Arbuscular Mycorrhizal Fungi (AMF) and Fermented Organic Manure on Yield and Quality of Forage Sorghum (*Sorghum Bicolor* L. Moench) Var. Panarunder Saline Conditions

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Abstract

A field experiment was conducted over two years (2015\2016) at Hada Al-Sham Experimental Research Station of King Abdulaziz University in Jeddah, Saudi Arabia. This study aims at evaluating the effect of AMF (arbuscular mycorrhizal fungi) applied separately and in combination with fermented poultry and cow manures on forage yield and quality of forage Sorghum var. Panar grown in saline soil and irrigated with saline water. Results showed that significant differences were reported for the AMF combined with both poultry and cow manures over the control treatment in all parameters studied including plant height, number of leaves per plant, leaf area, leaf firing ratio, yield and nutritive value. The effectiveness of the treatments were as follows: AMF mixed with both poultry and cow manures (PCM-AMF)> AMF mixed with poultry manure (PM-AMF)> AMF mixed with cow manure (CM-AMF)> AMF alone(AMF)> control(S) but for leafing firing ratio the order was reversed. The increments in dry forage yield were118, 82, 64, and 26% for PCM-AMF, PM-AMF, CM-AMF and AMF, respectively in comparison to the control. The nutritive value of the forage was significantly improved by the fungal and poultry manure treatments. It can be concluded from the results of this study that organic manures, particularly poultry and cow manures, mixed with Arbuscular Mycorrhizal Fungi had positive effects in increasing forage yield and improving quality of the cereal forage Panar under Soil and water salinities prevailing in arid regions. To overcome plant production limitations in saline arid conditions, it is recommended to use organic manures and mycorrhizal fungi, as they are relatively cheaper, available and effective.

Keywords: Arbuscular Mycorrhizal Fungi; Cereal Forage Yield; Quality; Cow Manure; Poultry Manure; Salinity

Introduction

Arid lands are characterized with fragile ecosystems, low crop productivity because of limited good quality irrigation water and non-sustainable farming systems. About 7% of the global land surface is covered with saline plant habitat [1]. Moreover, about 20% of the irrigated land has suffered from secondary salinization and 50% of irrigated schemes are affected by salt [2]. The Kingdom of Saudi Arabia entirely lies within arid zone with an annual rainfall ranging from 00 to 100 mm/annum. This limited rainfall coupled with high evaporation rates allow limited cultivable pockets in valleys of the western region, which mostly depends on saline irrigation that hinder growth and productivity of plants.

Organic farming has become one of the fastest growing segments of agriculture throughout the world because in conventional agriculture system use of chemicals has worried people about food quality, sustainability and other environmental consequences while organic agriculture assures high-quality food, sustainability and protects the environment [3]. Saudi Arabia soils have low soil organic matter. It is well known that enrichment of organic matter reduces salinity effect and increases moisture conservation and as result stimulates crop growth and quality [4,5]. The use of organic fertilizers, particularly poultry and farmyard manures, are known to benefit soils under such adverse environment through improving soil physical and chemical properties, thereby enhancing crop productivity [6]. Several researchers pointed out that organic manure help in conserving cropping systems through recycling of nutrients [7,8]. Moreover, reported that addition of organic manures with crop residues led to an increase in available phosphorus in soil compared to the control [9]. On the other hand, the use of inorganic fertilizers, particularly under saline condition, has not been helpful and is often associated with reduced crop yield, cause soil acidity and nutrient imbalances [10,11].

Arbuscular mycorrhizal fungi (AMF) have been shown to decrease plant yield losses in saline soils on tomato; on Coriandrum sativum; on subterranean clover; on maize [12-16]. It was reported that AMF increased yield of tomato in saline soils by 32% compared to non-inoculated plants [13]. Arbuscular mycorrhizal fungi (AMF) is known to increase root density thereby assists in root absorption to moisture and nutrients especially phosphorous. It was reported that P and Ca concentrations in plants inoculated with AMF were significantly greater than those non-inoculated; whereas Na concentration decreased indicating, that AMF controls absorption of Na below the toxicity level [17].

The aim of this research was to evaluate the effects of addition of fermented cow and poultry manures alone and in combination with each other in addition to the mycorrhizal fungi (AMF) as seed pellets for Panar cereal forage to serve as soil conditioner and nutrient suppliers in a saline arid condition as the experiment was conducted on typical natural saline site, at Hada Al-Sham Experimental Research Station of King Abdulaziz University120 km north–east of Makkah in western Saudi Arabia.

Materials and Methods

An experiment was conducted at Hada Al-Sham Research Station over two successive seasons during 2015/16. The experiment included a hybrid forage Sorghum (Sorghum bicolor L Moench.) cv. Panar as a test crop. The treatments consisted of:

1. Arbuscular Mycorrhizal Fungi (AMF)
2. Fermented poultry manure applied at a rate of five tons ha-1- plus AMF (PM-AMF)
3. Fermented cow manure applied at a rate of five tons ha-1 plus AMF (CM-AMF)
4. Fermented poultry and cow manure combined at a rate of two and half tons ha-1 of each plus AMF PCM-AMF
5. Control: no manure or mycorrhizae were added

The poultry and cow manures were fermented for six weeks before use to eliminate all weed seeds and pathogens present in the manure. Thereafter, it was incorporated into soil before planting. In addition, seeds were pelleted with the manure and the fungi according to treatments.

Indigenous AMF spores were isolated by the sieving and decanting method [18]. Isolated spores were multiplied and maintained on Panar forage Sorghum grown in pots under controlled environment for six months prior to field experiment. Immediately before commencing field experiment, spores and hyphae were collected from pots and inoculated to the Panar seeds of the field experiment. The inoculum contained about 100-120 infectious AM propagules per 10 g of soil.

Soil Analysis

Ten random soil samples from the experimental site were analyzed for the chemical properties of the soil using an auger to a depth of 30 cm. Chemical properties of soil are presented in Table 1.

Ec	pH	%OM	N(gkg-1)	P(gkg-1)	K(gkg-1)	Ca(gkg-1)	Mg(gkg-1)	Na(gkg-1)
5.5	7.72	0.045	0.02	0.045	0.035	0.283	0.269	0.35

Table 1: Chemical properties of the experimental soil.

Chemical Analysis of Poultry and Cow Manures

At the end of fermentation period, both cow and poultry manures were chemically analyzed as shown in Table 2.

	N (gkg-1)	P (gkg-1)	K(gkg-1)	C (gkg-1)	C/N
Poultry manure	16.35	3.9	23.95	255	16.1
Cow manure	7.58	1.02	12.8	87	13.1

Table 2: Chemical analysis of poultry and cow manures.

Irrigation Water

Bore hole water was the source of irrigation which was pumped via plastic pipes to the experimental plots. Experimental plot size was 2×2 meters. Salinity of the irrigation water was 3500TDS (ppm). Irrigation was applied lightly every other day to avoid salt crust on soil surface.

The growing season in western Saudi Arabia usually starts from October to April, as before and after these months, temperature and humidity are too high (temperature above40◦C and relative humidity above 80%) coupled with high soil and irrigation water salinity that does not permit good crop growth. Rainfall is usually rare and ranges between 00 to 100 mm/annum occurring during November and December.

The field experiment started on 1st of October and terminated at end of April during the two consecutive seasons. Three harvests of the forage were available each year. Plants were cut at 50% bloom stage.

The following growth and yield parameters were taken during the course of the study:

Plant Height

Plant height of the Panar forage was measured at each harvest during each season. Measurements were taken from the tip of the flag leaf to the soil surface. Ten random plants were randomly taken from each treatment for this purpose.

Total Number of Leaves per Plant

Total number of leaves per plant was counted at first and third harvests in each season. Ten random plants were taken from each treatment for this parameter.

Leaf Firing Ratio

Number of dead leaves per plant was counted for the same plants used for counting total number of leaves per plant to calculate leaf-firing ratio for each treatment. Leaf firing ratio was calculated using the formula: Leaf firing ratio= number of dead leaves per plant/ total number of leaves per plant [19]. This parameter gives an indication on how long leaves remain green and healthy on plants before senescence, which is an important character in forage quality and quantity.

Total Leaf Area per Plant (cm^{-2})

Ten random plants at 1st and 3rd harvests in each treatment were used for calculating total leaf area per plant. Measurements of the entire length of leaf blade and the maximum width were taken for each leaf of the ten random plants. Then leaf area was calculated according to Kemp using the formula [19]:

$$\text{Leaf area} = K \times L \times W;$$

Where K is a constant in cereals equals to 0.75, L is maximum length of the blade and W is maximum width of the blade. From this total leaf, area per plant was calculated by multiplying leaf area times the number of leaves per plant.

Forage Fresh and Dry Yields (tons/ha)

The entire plot (2 × 2 m) was harvested and weighed to get forage fresh yield, whereas a sample was taken from each plot, oven dried at 75 °C for 48 h. until a constant weight was reached to obtain dry yields. Both fresh and dry yields were transformed from kg ha^{-1} into tons ha^{-1}.

Forage Nutritive Value

Proximate analysis for plant tissues to determine the nutritive value of the forages was performed according to

AOAC [20]. It was performed on the final cut each season to reduce cost and differences were expected between treatments rather than the cuts.

Experimental Design and Data Analysis

A randomized complete block design (RCBD) with three replications was used and analysis of variance (ANOVA) was performed on data according to Steel, et al [21]. Means of treatments were compared according to the LSD method.

Results

Soil and Manure Chemical Analysis

Result of soil analysis of the experimental site is presented in Table 1. Organic matter, nitrogen, phosphorus and potassium contents of the experimental soil were relatively low, while Na, Mg and Ca contents were high than optimal situations. The electric conductivity was 5.5. It is worth mentioning here that the irrigation water salinity was 5000ppm.

Chemical analysis of the fermented organic manures indicated that poultry manure contained about three folds nutrients compared to cow manure (Table 2).

Growth Parameters

Plant Height: Significant (*P≤0.05*) differences were reported for plant height in all cuts of the two growing seasons (Table 3). Inoculated plants with AMF alone or mixed with poultry and/or cow manure produced significantly taller plants than the control. Tallest plants were always recorded for the PCM-AMF compared to other treatments throughout the two seasons. The ranking order of treatments with respect to plant height was PCM-AMF > PM-AMF > CM-AMF > AMF > Control.

Treatments.	1st	season		2nd	season	
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
control	120.33d	133.00d	128.33d	130.33d	85.00e	62.00d
SM	140.33c	147.66c	139.33cd	144.00c	93.66 d	69.66c
SMPM	159.33b	167.00b	156.66b	160.66ab	96.66c	82.00b
SMCM	155.00b	153.66c	147.00bc	150.66bc	113.66ab	80.33b
SMPMCM	169.67a	186.66a	168.00a	170.66a	120.66a	95.00a
LSD at 5% prob.	10	10.01	10.5	13.42	8.6	7
CV%	10.55	4.75	5.75	4.71	9.97	10.1

Figures followed by same letter (s) within each column are not significantly different using LSD test at 0.05 level of probability.
Control: No treatment
AMF: Arbuscular Mycorrhizal Fungi
PM-AMF: Poultry manure-AMF
CM-AMF: Cow manure-AMF
PCM-AMF: Poultry and cow manures' mixture-AMF
Table 3: Effect of treatments on Panar plant height.

Number of Leaves per Plant: Significant (*P≤0.05*) differences between treatments were reported for number of leaves per plant throughout the experimental period (Table 4). The highest number of leaves per plant was always recorded for PCM-AMF compared to other treatments. The same ranking order reported for plant height was repeated for number of leaves per plant.

Treatments	1st	season	2nd	season
	1st cut	3rd cut	1st cut	3rd cut
control	7.61c	6.15d	6.66b	6.00b
SM	8.35bc	7.95c	7.33b	7.00ab
SMPM	9.14b	9.94b	9.66a	8.00ab
SMCM	9.95b	8.85c	8.00b	7.66ab
SMPMCM	10.96a	10.97a	10.33a	9.66a
LSD at 5% prob.	0.83	1	1.55	2.88
CV%	9.75	12.5	9.84	19.99

Figures followed by same letter (s) within each column are not significantly different using LSD test at 0.05 level of probability
Control: No treatment
AMF: Arbuscular Mycorrhizal Fungi
PM-AMF: Poultry manure-AMF
CM-AMF: Cow manure-AMF
PCM-AMF: Poultry and cow manures' mixture-AMF
Table 4: Effect of treatments on Panar number of leaves per plant.

Total Leaf Area per Plant: Effect of treatments on total leaf area per plant is presented in Table 5. The highest total leaf area per plant was scored by PCM-AMF and it was significantly different from other treatments throughout the experimental period. The fungi with cow manure always exceeded the fungi with poultry manure although they were significantly different in the second season only. The least total leaf area per plant was always recorded for the control treatment.

Treatments	1st	season	2nd	season
	1st cut	**2nd cut**	**1st cut**	**2nd cut**
control	491c	395c	437.0e	310.0d
SM	576c	410c	531.0d	399.3c
SMPM	680b	715b	605.0c	670.6c
SMCM	720b	845b	729.0b	803.3b
SMPMCM	1230a	1012a	1112.5a	896.3a
LSD at 5% prob.	100	132	71.5	85
CV%	15.6	13.8	18.17	14.29

Figures followed by same letter (s) within each column are not significantly different using LSD test at 0.05 level of probability.
Control: No treatment
AMF: Arbuscular Mycorrhizal Fungi
PM-AMF: Poultry manure-AMF
CM-AMF: Cow manure-AMF
PCM-AMF: Poultry and cow manures' mixture-AMF
Table 5: Effect of treatments on Panar total leaf area per plant (cm^{-2}).

Leaf Firing Ratio: Results of leaf firing ratio as affected by treatments are presented in Table 6. Significant (*P≤0.05*) differences between treatments were observed for leaf firing ratio. An opposite trend was observed compared to the previous mentioned parameters for leaf firing ratio since the control treatments significantly outscored other treatments. The ranking order for leaf firing ratio was Control > AMF > CM-AMF > PM-AMF > PCM-AMF.

Treatments	1st	season	2nd	season
	1st cut	2nd cut	1st cut	2nd cut
control	0.39a	0.43a	0.37a	0.42a
SM	0.31b	0.35b	0.28b	0.31ab
SMPM	0.29b	0.25c	0.27bc	0.26b
SMCM	0.24c	0.20c	0.22cd	0.21b
SMPMCM	0.20c	0.18c	0.21d	0.20b
LSD at %5 prob.	0.04	0.06	0.056	0.13
CV%	12.71	15.6	10.96	20.07

Figures followed by same letter (s) within each column are not significantly different using LSD test at 0.05 level of probability.
Control: No treatment
AMF: Arbuscular Mycorrhizal Fungi
PM-AMF: Poultry manure-AMF
CM-AMF: Cow manure-AMF
PCM-AMF: Poultry and cow manures' mixture-AMF
Table 6: Effect of treatments on Panar leaf firing ratio.

Forage Yield

Fresh Yield: Forage fresh yield (Table 7)was significantly (*P≤0.05*) affected by treatments in all cuts throughout the experimental period. Higher forage fresh yield was always recorded for PCM-AMF compared to other treatments. The ranking order for treatments in respect to fresh yield was PCM-AMF› PM-AMF› CM-AMF›AMF›Control, with the exception of 3rd cut in season one and 1st cut in season two when cow manure exceeded poultry manure and the difference was not significant between the two treatments. Calculating the overall means of treatments for the two seasons, the increments in forage fresh yields were 101, 80, 66, and 21%for PCM-AMF, PM-AMF, CM-AMF and AMF, respectively in comparison to the control treatment.

Treatments	1st	season		2nd	season	
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
control	6.76c	11.00c	8.13d	17.66b	6.76e	7.53e
SM	8.10c	12.40c	12.80c	19.00b	8.60d	9.53d
SMPM	18.66a	24.43a	16.16b	21.50ab	9.86c	13.33b
SMCM	13.86b	19.66b	16.73ab	21.80ab	11.70b	10.86c
SMPMCM	21.43a	25.76a	18.96a	23.80a	10.80a	16.20a
LSD at %prob.	7.07	4.56	2.55	2.1	0.89	1.2
CV%	13.04	13.05	9.33	10.5	13	12

Figures followed by same letter (s) within each column are not significantly different using LSD test at 0.05 level of probability.
Control: No treatment
AMF: Arbuscular Mycorrhizal Fungi
PM-AMF: Poultry manure-AMF
CM-AMF: Cow manure-AMF
PCM-AMF: Poultry and cow manures' mixture-AMF
Table 7: Effect of treatments on Panar fresh yield (ton ha^{-1}).

Dry Yield: The effect of treatments on dry forage yield is presented in Table 8. Significant (*P≤0.05*) differences were recorded between treatments for dry yield. Similar to fresh yield, the ranking order for dry yield was PCM-AMF >PM-AMF>CM-AMF > AMF >Control, with the exception of 3rd cut in season one and 1st cut in season two when cow manure exceeded poultry manure and the difference was not significant between the two treatments. The PCM-AMF treatment produced the highest dry yield, whereas the control treatment produced the lowest dry yield throughout the two seasons. Increments in dry yields were 118, 82, 64, and 26% for PCM-AMF, PM-AMF, CM-AMF and AMF, respectively in comparison to the control treatment.

Treatments	1st	season		2nd	season	
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
control	3.66c	5.26c	3.47b	7.00c	2.76d	2.43c
SM	4.46c	6.50bc	4.98b	8.16b	3.80c	2.86c
SMPM	8.60b	11.66a	7.10a	8.30b	4.60ab	4.53ab
SMCM	6.90b	8.56b	8.30a	8.66b	4.26bc	3.86b
SMPMCM	11.80a	12.80a	8.96a	9.70a	5.20a	5.10a
LSD at 5%prob.	1.9	2.6	2.07	1	0.7	0.86
CV%	14.3	15.48	12.76	13.1	9.36	12.52

Figures followed by same letter (s) within each column are not significantly different using LSD test at 0.05 level of probability.
Control: No treatment
AMF: Arbuscular Mycorrhizal Fungi
PM-AMF: Poultry manure-AMF
CM-AMF: Cow manure-AMF
PCM-AMF: Poultry and cow manures' mixture-AMF
Table 8: Effect of treatments on Panar dry yield (tonha^{-1}).

Forage Nutritive Value

Results of proximate analysis for plant tissue of the Panar forage are presented in Table 9. Significant (*P≤0.05*) differences were reported for all parameters analyzed in both seasons. Maximum crude protein, Ca, P, and Mg percentages were recorded for the fungi mixed with poultry and cow manures (PCM-AMF), whereas the least percentages were recorded for the control treatment. On the other hand, the opposite was true for the crude fiber percent where the control treatment scored the highest CF percent in the first season in comparison to treated plots.

Treatments		1st	season				2nd	Season		
	%CP	%CF	%Ca	%P	%Mg	%CP	%CF	%Ca	%P	%Mg
control	4.60d	28.8a	0.24c	0.35d	0.35d	6.2c	28.6b	0.31b	0.34d	0.43c
SM	4.62d	28.6b	0.24c	0.60c	0.52c	9.2ab	30.8a	0.36a	0.58c	0.61b
SMPM	5.52b	26.7c	0.27ab	0.74a	0.70a	9.5a	30.9a	0.38a	0.72a	0.91a
SMCM	5.05c	26.7c	0.26b	0.70b	0.61b	9.3a	30.8a	0.37a	0.68b	0.61b
SMPMCM	5.94a	26.4d	0.28a	0.75a	0.75a	9.5a	30.9a	0.38a	0.73a	0.92a
LSD at 5%prob.	0.03	0.1	0.01	0.02	0.05	0.2	0.1	0.15	0.02	0.17
CV%	2.4	1.5	5.2	2.3	4.3	5.5	2.6	3.6	2.5	10.6

Figures followed by same letter (s) within each column are not significantly different using LSD test at 0.05 level of probability.
Control: No treatment
AMF: Arbuscular Mycorrhizal Fungi
PM-AMF: Poultry manure-AMF
CM-AMF: Cow manure-AMF
PCM-AMF: Poultry and cow manures' mixture-AMF
CP= crude protein CF= crude fiber
Table 9: Effect of treatments on chemical analysis of plant tissues.

Discussion

Growth Parameters

Growth parameters studied included plant height, number of leaves per plant, total leaf area per plant, and leaf firing ratio. All these growth parameters contribute to forage yield and quality. In all growth parameters, with the exception of leaf firing ratio, mycorrhizal addition with poultry and cow manures out yielded other treatments in plant height, leaf number and leaf area. Leaf firing ratio decreased with the fungus and manures addition .This is a good character achieved via applications of the manure and the fungus since low leaf firing ratio means more green leaves remain attached to the plant at harvest, which would improve both quality and quantity of the forage. This was expected as the manure improved physical and chemical properties of soil to facilitate water infiltration, nutrient uptake and gas exchange between soil and air and reduces salinity effect on plant growth [4,5,22]. Moreover, the organic manures (cow and poultry manures) contribute to essential nutrients that improved plant growth particularly in such saline soils [6]. Several researchers pointed out that organic manure helps in conserving cropping systems through recycling of nutrients and increases available phosphorus in soil [7,9]. It is worth mentioning here that analysis of poultry manure showed three folds nutrients than those in cow manure. This was reflected in the results where AMF mixed with poultry manure (PM-AMF) significantly exceeded AMF mixed with cow manure (CM-AMF) in all parameters studied except leaf firing ratio. This could be explained on the basis that treatments containing manure and fungi provided enough nutrients to keep growing leaves healthier and green until harvesting time and the opposite was true for the control treatment.

These results are in line with those reported by, working on tomato, Acacia auriculiformis, subterranean clover and on maize, respectively [12-16]. They reported that AMF reduces plant yield losses in saline soils. It was reported that AMF increased yield of tomato in saline soils by 32% compared to non-inoculated plants [12]. Arbuscular mycorrhizal fungi (AMF) is known to increase root density via root proliferation, thereby assists in root absorption to moisture and nutrients especially phosphorous [17]. It was reported that P and Ca concentrations in plants inoculated with AMF were significantly greater than those non-inoculated; whereas Na concentration decreased indicating, that AMF controls absorption of Na below the toxicity level [17].

Forage Yield

Forage fresh and dry yields were increased significantly under mycorrhizal treatment with the manures in comparison to the control. The mycorrhizal addition to poultry manure (PM-AMF) and the combination of poultry and cow manures with AMF (PCM-AFM) out yielded other treatments with respect to forage yield (fresh and dry). This was a reflection of results on growth parameters (plant height, number of leaves plant and leaf area) which all contributed to forage yield. The highest forage fresh yield was recorded for PCM-AMF in five out of six cuts and in all cuts for the dry weight. The ranking order of treatments for both fresh and dry yields was PCM-AMF >PM-AMF>CM-AMF > AMF >Control in four out of six cuts, which could be an indication of the effectiveness of combining the fungal mycorrhizae with organic manures to mitigate the effect of salinity on plant production. The more effectiveness of PCM-AMF may be because the mixture is less saline compared to sole poultry manure, as well as it provides the necessary nutrients [22].

Forage Nutritive Value

Organic manures (particularly Poultry manure) and mycorrhizal addition improved forage quality in terms of crude protein, crude fiber and nutrients in plant tissues especially phosphorus content. The highest CP, Ca, P and Mg was consistently recorded for PCM-AMF treatment ,whereas the lowest value was recorded for the control treatment in both seasons The ranking order for treatments regarding nutritive value were similar to those recorded for fresh and dry yields (PCM-AMF >PM-AMF>CM-AMF > AMF >Control).Results of manure analysis showed that poultry manure contained three folds nutrients than cow manure. Arbuscular Mycorrhizal Fungi (AMF) is known to increase root density, therefore assists in root absorption to moisture and nutrients in soil, especially phosphorous. This in line with results reported by Bhoopander, et al who stated that P and Ca concentrations in plants inoculated with AMF were significantly greater than those non-inoculated; whereas Na concentration decreased indicating that AMF controls absorption of Na below the toxicity level [17].

Conclusion and Recommendation

It can be concluded from the results of this study that organic manures, particularly poultry and cow manures, mixed with Arbuscular Mycorrhizal Fungi had positive effects in increasing forage yield and improving quality of the cereal forage Panar under Soil and water salinities prevailing in arid regions.

To overcome plant production limitations in saline arid conditions, it is recommended to use organic manures and mycorrhizal fungi, as they are relatively cheaper, available and effective.

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