

Effect of Intercropping System on the Quality and Quantity of Runner Bean (*Phaseolus coccineus* L.)

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Abstract

Field experiments were conducted two consecutive years, in order to investigate runner bean (*Phaseolus coccineus* L.) cultivated in pure crop system and in intercropping system. To achieve the desired aim, we set the following objectives: study of runner bean plant phenology, study of assimilating pigments content in the runner bean leaves, agro productivity study of runner bean crop, yield of runner bean crop, study of chemical composition of runner bean dry seeds. The studied experimental factor was the trellis system with six variants: (V1) - trellis, with double rows, on individual string, (V2) - trellis, with a single row, on individual string, (V3) - trellis, in a single row, on synthetic net (17 cm mesh), (V4) - intercropping with common maize plants, (V5) - intercropping with sunflower plants, (V6) - intercropping with Jerusalem artichoke plants. Runner bean yield ranged from 3,610 kg ha⁻¹ (V2) to 1,684 kg ha⁻¹ (V6) (first year) and from 3,170 kg ha⁻¹ (V2) to 1,189 kg ha⁻¹ (V6) (second year). Runner bean dry seeds contain: crude protein 22.65% (V6) - 25.47% (V3), crude fat 1.91% (V6) - 2.16% (V4), crude fibre 5.41% (V4) - 5.81% (V5) and nitrogen free extractive substances 62.11% (V3) - 64.92% (V4). The best variant of trellis for runner bean crop was the single row trellis system on individual string and the best intercropping system was intercropping with sunflower.

Keywords: chemical composition, chlorophyll content, crop systems, grain yield, phenotypic evaluation

Introduction

Runner bean (*Phaseolus coccineus* L.) is a herbaceous, out-crossing species, propagated, usually, by seeds (Popa, 2010). In the centre of origin (Mexico and Central America) it is perennial, while in temperate conditions (including Romania), it behaves as an annual species, because the plants, including the root system, do not withstand at low temperatures during wintertime (Rodiño *et al.*, 2007; Labuda, 2010; Popa, 2010). As a cultivated plant, *P. coccineus* is on the third place, as importance, worldwide, in the *Phaseolus* group, after *P. vulgaris* and *P. lunatus* (Santalla *et al.*, 2004; Rodriguez, 2013), but in Romania it is on the second place, being well known and sometimes being confused with the common bean (Popa, 2010). Being a vegetable species of warm and humid climate, under the forest steppe conditions from Romania, the crop falls under soil drought, atmospheric drought, as well as excessive high temperatures (Popa, 2010).

Therefore, in order to achieve efficient and stable yields, it is necessary to develop a cultivation system in which, through technological practices, unfavourable environmental conditions can be corrected (Hamburdă *et al.*, 2013). These practices should also provide a trellis support in a cheap and efficient culture

system for the climbing forms. Such a system of cultivation could be represented by interleaving runner bean plants with other crops, which is the "intercropping" system (Hamburdă *et al.*, 2014).

Intercropping is known for thousands of years and is the system in which, on the same land and in the same time, two or more species are cultivated (Ofori and Stern, 1987; Anil *et al.*, 1998). This culture system provides mutual favourable conditions for the species that are grown (Mousavi and Eskandari, 2011). The mutual favourability of the conditions is appreciated by a better plant growth and development, a superior quantitatively and qualitatively yield and a higher economic efficiency. Moreover, in the ecosystem, intercropping ensures the preservation and improvement of microorganisms' diversity, useful flora and fauna, which, frequently, have a positive impact on crop yields (Vandermeer, 1995; Butnariu *et al.*, 2014; Hamburdă *et al.*, 2015).

In terms of phenology, it is known that runner bean has a relatively long growing season, compared to common bean, reaching up to 130-140 days (Hadjichristodoulou, 1990; Popa, 2010; Munteanu *et al.*, 2013). The yield amount, just like its quality, is conditioned by genotype, environment and interaction

between these factors (Labuda, 2010). For runner bean, as well as other species of the same group, the amount of yield is directly determined by the number of flowers, fruits and seeds per plant and these are correlated with the photosynthetic activity of the plant, in accordance with the environmental conditions (Labuda, 2010).

Runner bean food value is determined by complex chemical composition, with nutritional, energetic, catalytic and mineralizing value (Aremu *et al.*, 2010; Labuda, 2010; Popa, 2010). Vegetable products of this species are sources of complex carbohydrates, protein and dietary fibre, having significant amounts of vitamins and minerals and high energetic value (Tharanathan and Mahadevamma, 2003). Protein contents in dried grains range from 17% to 40%, contrasting with 7-13% of cereals, and being equal to the protein contents of meats (18-25%) (Genovese and Lajolo, 2001; De Almeida Costa *et al.*, 2006).

The purpose of this paper was to present the behaviour of runner bean in a pure crop system and in intercropping system, with common maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.) and Jerusalem artichoke (*Helianthus tuberosus* L.). To achieve the desired aim, we set the following objectives: study of runner bean plant phenology; study of assimilating pigments content in the runner bean leaves; agro productivity study of runner bean crop; yield of runner bean crop; study of chemical composition of runner bean dry seeds (quality of the harvest).

Materials and Methods

Location and experimental conditions

The experiment was organized during two consecutive years (2014 and 2015) in the experimental field of the vegetable research laboratory of the Adamachi Experimental Station at the University of Agricultural Sciences and Veterinary Medicine (UASVM) Iași, located in North-Eastern Romania (47°10'43"N and 27°37'14"E). The climate is temperate continental, with an average annual temperature of 9.6 °C (49.3 °F) and a total average rainfall of 521 mm per year. In 2014 (during January-August) was recorded 11.9 °C temperature, 70.5% humidity and 343.4 mm rainfalls. In 2015 (during January-August) was recorded 19.3 °C temperature, 54.4% humidity and 156.8 mm rainfalls (Table 1). In terms of morphological and systematic soil conditions, the soil is classified as chernozem (Cz), with an average supply of nutritive elements, 3.8% organic matter and a pH of 5.8.

Biological material and experimental design

The biological material consisted of runner bean seeds ('C3' local population, with white seeds), common maize seeds ('Flato' F1 hybrid), sunflower seeds ('Tristan' F1 hybrid) and Jerusalem artichoke tubers ('Topstar' cultivar). The experiment was organized using a randomized block design with three

replication, where each replication was covering an area of 6 square. The studied experimental factor was the trellis system with six variants:

- (V1) = trellis, with double rows, on individual string,
- (V2) = trellis, with a single row, on individual string,
- (V3) = trellis, in a single row, on synthetic net (17 cm mesh),
- (V4) = intercropping with common maize plants,
- (V5) = intercropping with sunflower plants,
- (V6) = intercropping with Jerusalem artichoke plants.

On each replication plot, two rows of nests of two plants were placed, spaced at 1.0 m, and between nests and plants, on the row, there was a distance of 0.4 m. In the variant V1, the distance between the two rows on the same trellis was 0.4 m. The distance between common maize plants, the sunflower plants and Jerusalem artichoke plants was 0.8 m. For runner bean pure crop (V1, V2 and V3 variants), the support system consisted of a trellis composed of reinforced concrete pillars and a steel wire of 2.0 mm diameter, located on their upper end. The crop was established in the first part of May, when the average temperature in the soil, at 3-5 cm, has a value of 10-12 °C, by direct sowing of three runner bean seeds per nest and two seeds per nest for common maize and respectively, sunflower. At the time of emergence, two runner bean plants and one plant of common maize or sunflower have been left in each nest. As for the Jerusalem artichoke, the tubers were planted in pairs per nests and at the emergence time only two stems per nest were allowed. Sowing of common maize and sunflower, respectively planting Jerusalem artichokes, were made about two weeks before sowing the runner bean. The experiment was conducted in accordance with technological recommendations arising from the specialized literature for runner bean and for associated plants (Popa, 2010).

Assessment of experimental variants

Experimental variants were evaluated based on observations and biometric measurements: plant phenology, assimilating pigments content, dry matter (DM), crude ash (CA), organic matter (OM), crude protein (CP), crude fat (EE), crude fibre (CF), nitrogen free extractive substances (NFES) and agro productivity.

Plant phenology was assessed by recording the main phenological phases of the crop: the period from sowing to emergence; emergence-first real trifoliate leaf; emergence-first flowers; emergence-first pods; emergence-seed maturation; emergence-end of vegetation.

Content in assimilating pigments was determined in runner bean leaves, using the spectrophotometric method, according to the protocol described by Lichtenthaler and Buschmann (2001). Leaf samples were taken from the upper third of the plant, corresponding to the area with young pods (green) in two moments: the first moment, when the plants were aged 58 days and one-two pods were formed in the upper third of the plant; the second moment, when plants had 107 days, toward the end of the vegetation period. Through the spectrophotometer, the absorbance at 661.6 nm, 644.8 nm and 470 nm was measured, which correspond, respectively, to chlorophyll *a*, chlorophyll *b*, and carotenoids. After absorbance detection, the following formulas were used to determine quantity, in mg/g:

Chlorophyll *a*: $CHLa = (((11.24 \times \lambda_{661.6} - 2.04 \times \lambda_{644.8}) \times 10) / M) / 1000;$

Table 1. Meteorological conditions of the two experimental years

Experimental Year	Temperature (°C)	Humidity (%)	Rainfalls (mm)
First Year (2014)	11.9	70.5	343.4
Second Year (2015)	19.3	54.4	156.8

Chlorophyll *b*: $CHLb = (((20.13 \times \lambda_{644.8} - 4.19 \times \lambda_{661.6}) \times 10) / M) / 1000$;

Carotenoids = $((1000 \times \lambda_{470} - 1.9 \times CHLa - 63.14 \times CHLb) / 214 \times 10) / M / 1000$, where, M = mass of fresh plant material, CHLa = chlorophyll *a*, CHLb = chlorophyll *b*.

Leaf dry matter was determined gravimetrically by drying in an oven at a temperature of 105 °C (AOAC, 1990).

Agro productivity was evaluated by determination of: the number of pods per plant, number of grains per pod, number of seeds per plant, mass of one thousand seeds (MTS) and the amount of seeds per hectare (kg ha^{-1}).

The chemical composition was determined by the official methods of the Association of Official Chemists (1990). Percent protein was calculated as $\% N \times 6.25$.

Data processing and statistical analysis

The statistical significance was determined by one-way analysis of variance (ANOVA) analysis. 'Duncan's Multiple Range Test', one of the multiple comparison tests, was used when the difference between groups was found to be statistically significant (Ardelean et al., 2007). The measure of statistical significance was a P value below 0.05 ($P < 0.05$). The data were presented on the basis of mean \pm standard deviations

Results and Discussion

Runner bean plant phenology

Morphological and physiological plant development, marked by phenophases of plant development, was not obviously influenced by the trellis system. Regarding the phenological characterization, the period from sowing to emergence was about seven to ten days, the period from emergence to appearance of the first real trifoliate leaves was

around six to seven days, the period from emergence to the first flowers was about 32-35 days, the period from emergence to the first pod was about 67-70 days, the period from emergence to seed maturation was around 115-120 days and the period from emergence to the end of the vegetation period was around 130-140 days (Table 2).

Among the studied variants, runner bean plants interleaved with Jerusalem artichokes had a slight delay of one-three days for the phenophases of the vegetation period, which could be due to delay caused by shading from Jerusalem artichoke plants, as well as the higher plant densities at unit surface (10 plants/square), respectively, the shading effect, in favour of phenological processes delay, also influencing the yield (Reynolds et al., 2007). The results that were obtained in pure crop are consistent with those reported by Hadjichristodoulou (1990), Popa (2010) and Munteanu et al. (2013).

Assimilating pigments content in the runner bean leaves

The results of the content of runner bean leaves pigments are shown in Tables 3 and 4. At first measurement, the chlorophyll a/b ratio ranged from 2.39 mg/g f.w. at variant V6 to 2.76 mg/g f.w. at variant V1. It should be noted that, in the intercropping system, the plants' chlorophyll concentrations are higher than those grown in pure system. In this case, the shade contributes to increasing the concentration of chlorophyll in the leaves, as a result of an adjustment mechanism of plants to lower luminance, high levels of chlorophyll being typical for ombrophile plants. Similar results were obtained by Makus (2001) at two cultivars of *Phaseolus vulgaris* L., Strike and Carlo. Chlorophyll *b* is very abundant in shaded leaves, to improve the ability of chloroplasts to capture light. However, yield components have been significantly affected by the shading in the case of runner bean interleaved with common maize and Jerusalem artichoke. At the second measurement, there has been a decrease in the

Table 2. Phenological characters for runner bean crop (number of days)

Variant	Sowing (emergence)	Emergence (first real trifoliate leaf)	Emergence (first flowers)	Emergence (first pods)	Emergence (seed maturation)	Emergence (end of vegetation)
V1	7-10	6-7	32-34	67-70	115-120	130-137
V2	7-10	6-7	32-34	67-70	115-120	130-137
V3	7-10	6-7	32-34	67-70	115-120	130-137
V4	7-10	6-7	32-34	67-70	115-120	130-137
V5	7-10	6-7	32-34	67-70	115-120	130-137
V6	8-10	7-8	32-35	68-71	117-121	135-140

Table 3. The content of assimilating pigments at the first measurement (mg/g f.w.)

Variant	DM	CHLa	CHLb	C+X	Σ pigments	CHLa/CHLb	(CHLa/CHLb)/(C+X)
V1	2.21	1.87	0.68	0.68	3.23	2.76	3.73
V2	2.67	1.76	0.65	0.64	3.04	2.71	3.76
V3	2.32	1.48	0.54	0.55	2.56	2.72	3.68
V4	1.56	2.25	0.86	0.83	3.95	2.6	3.74
V5	1.58	2.18	0.82	0.81	3.81	2.64	3.68
V6	1.20	2.5	1.05	0.91	4.46	2.39	3.92
Average	1.92	2.00	0.76	0.73	3.50	2.63	3.75

Note: f.w. = fresh weight; DM = dry matter; CHLa = chlorophyll a; CHLb = chlorophyll b; C+X = carotenes and xanthophylls

Table 4. The content of assimilating pigments at the second measurement (mg/g f.w.)

Variant	DM	CHLa	CHLb	C+X	Σ pigments	CHLa/ CHLb	(CHLa/CHLb)/ (C+X)
V1	19.33	1.04	0.41	0.36	1.81	2.51	3.95
V2	17.88	0.95	0.37	0.31	1.63	2.59	4.2
V3	18.32	1.09	0.5	0.39	1.99	2.2	4.07
V4	20.09	1.15	0.46	0.41	2.02	2.51	3.91
V5	15.82	1.1	0.48	0.37	1.95	2.37	4.18
V6	17.21	1.43	0.61	0.49	2.53	2.35	4.11
Average	18.11	1.12	0.47	0.38	1.98	2.42	4.07

Note: f.w. = fresh weight; DM = dry matter; CHLa = chlorophyll a; CHLb = chlorophyll b; C+X = carotenes and xanthophylls

concentration of chlorophyll, mainly due to the decrease of chlorophyll *a* content, the chlorophyll *a/b* ratio being superior to the first measurement (ranged from 2.2 mg/g f.w. at variant V3 to 2.59 mg/g f.w. at variant V2). These aspects allow us to state that the yield decrease under the influence of shading can be explained by the lack of storage organs and the reducing number of pods per plant, and not by photosynthetic activity. In this case, the source (leaves)-sink (storage organs) relationship is affected. Research by Plaut *et al.* (1987) showed that, in the case of sink reduction, the plant recorded a blockage of assimilates in leaves, without them being stored. Similar results were obtained in our experiment.

Agro productivity of runner bean crop

Mass of one thousand seeds (MTS) is a very important factor to measure the yield. The quantity of seed per hectare for crop establishment is also influenced by this character. So it will be higher in variants with higher MTS (Popa, 2010).

The number of pods per plant ranged from eight at variant V6 to 17 at variant V3, with an average of 13 pods/plant. The number of seeds in pod had an average value of two-four, the lowest number of seeds in pod being recorded at variant V6 (two-three). The number of seeds per plant ranged from 35 at variant V6 to 66 at variant V2 and variant V5. MTS ranged between 976.63 g at variant V6 and 1,238.33 g at variant V3 (Table 5).

Similar results were obtained in studies of Sadeghi *et al.* (2011) and Cokkizgin *et al.* (2013) on common bean (*P. vulgaris* L.). Previous studies have shown that the MTS of runner bean ranges from 1,080 to 1,799 g (Hadjichristodoulou, 1990), and from 1,108 g to 1,308 g, with an average of 1,204.10 g (Popa, 2010). Changes in runner bean weight are due to variable genetic

Table 5. Elements of runner bean productivity

Variant	No. of pods/plant	No. of seeds/pod	No. of seeds/plant	MTS (g)
V1	10	3-4	46	1,053.13
V2	16	3-4	66	1,089.56
V3	17	3-4	53	1,238.33
V4	11	2-4	42	1,070.93
V5	17	3-4	66	1,006.46
V6	8	2-3	35	976.63
Mean	13	2-4	51	1,072.5

Note: MTS = mass of one thousand seeds

potential for this character (Popa, 2010).

Yield of runner bean crop

The result of the variance analysis showed that there is no difference among the variants for both years in the significance level of 1% (Table 6). According to the Duncan multiple group tests, variants were six different groups for both study years (Table 7). The highest yield was obtained from V2 and it was separated from the other variants in significance level of 5%. In the first experimental year, regarding the influence of the trellis system on the runner bean yield, the highest yield was obtained at variant V2 (3,610 kg ha⁻¹), followed by variants V5 (3,342 kg ha⁻¹), V3 (3,287 kg ha⁻¹), V1 (2,947 kg ha⁻¹), V4 (2,234 kg ha⁻¹) and the lowest yield was obtained at variant V6 (1,684 kg ha⁻¹). In the second experimental year the highest yield was obtained at variant V2 (3,170 kg ha⁻¹) followed by variants V5 (3,104 kg ha⁻¹), V3 (2,986 kg ha⁻¹), V1 (2,516 kg ha⁻¹), V4 (2,018 kg ha⁻¹) and the lowest yield was obtained at variant V6 (1,189 kg ha⁻¹). Comparing the experimental years there are very significant differences between variants, with a P value < 0.001. We noticed that in pure crop system was obtained the highest yield compared with the intercropping system.

In our previous studies (Hamburdă *et al.*, 2014), the highest yield was achieved by variant V3 (3,325 kg ha⁻¹) and the lowest by variant V6 (789 kg ha⁻¹), with a mean of 2,515 kg ha⁻¹. Yield differences obtained in the current study were attributed to weather conditions, 2015 being a drought year.

The chemical composition of runner bean dry seeds

The chemical composition of runner bean dry seeds from our experiment is presented in Table 8. We noticed that variant V4 recorded the highest value of organic matter, crude fat and nitrogen free extractive substances, and the highest crude protein content was obtained from V3 variant and it was separated from the other variants in significance level of 5%. The crude protein values of runner bean dry seeds are lower than other protein - rich foods, such as groundnut (*Arachis hypogaea* L.) (56.90%) (Conkerton, Ory, 1976) and soybean (*Glycine max* L.) (61.40%) (Rackis *et al.*, 1961). An adult man of 70 kg body weight requires 57 g protein i.e. 39.9 g of protein daily. This means that runner beans almost would supply the required protein, assuming complete protein absorption (Aremu *et al.*, 2006). Aremu *et al.* (2010) noted that *P. coccineus* had a crude fibre content of 10.5% higher than common legumes such as cowpea (*Vigna unguiculata* L.) (2.6%), Bambara groundnut (*Vigna subterranea*

Table 6. Mean squares from the variance analyses of the yield of runner bean in first and second experimental year

Source	d. f.a	Yield
First experimental year		
Blocks (B)	2	5.167 _{ns}
Variants (V)	5	1655430.8**
Error	10	150.167
Second experimental year		
Blocks (B)	2	684.667**
Variants (V)	5	1800698.9**
Error	10	22.067

Note: a = Degrees of freedom; ns = Non-significant; ** = Significant at the 1% of probability level ($P < 0.01$); * = Significant at the 5% of probability level ($P < 0.05$)

Table 7. Yield of runner bean ($P < 0.05$)

Variant	Yield of runner bean \pm SD (kg ha^{-1})	
	First Experimental Year	Second Experimental Year
V1	2,947 \pm 14.42d	2,516 \pm 14.18d
V2	3,610 \pm 13.75a	3,170 \pm 14.80a
V3	3,287 \pm 12.12c	2,986 \pm 6.08c
V4	2,234 \pm 9.85e	2,018 \pm 14.42e
V5	3,342 \pm 6.24b	3,104 \pm 7.94b
V6	1,684 \pm 8.72f	1,189 \pm 8.19f
Average	2,850.7	2,497.2
CV (%)	0.43	0.19
P value	<0.001	<0.001

Note: *Different letters indicate a significant difference of $P \leq 0.05$ (Duncan test); SD = standard deviations; CV = coefficient of variation

Table 8. Chemical composition of the dry matter of runner bean seeds (%)

Variant	DM	CA	OM	CP	EE	CF	NFES
V1	88.73 \pm 0.04	4.90 \pm 0.04	95.12 \pm 0.01	23.46 \pm 0.16	2.11 \pm 0.05	5.79 \pm 0.15	63.77 \pm 0.09
V2	88.60 \pm 0.04	5.05 \pm 0.03	94.84 \pm 0.03	23.40 \pm 0.32	1.95 \pm 0.07	5.54 \pm 0.10	63.95 \pm 0.28
V3	88.84 \pm 0.05	4.82 \pm 0.06	95.32 \pm 0.09	25.47 \pm 0.22	2.12 \pm 0.06	5.63 \pm 0.15	62.11 \pm 0.20
V4	89.24 \pm 0.03	5.02 \pm 0.02	95.56 \pm 0.02	23.08 \pm 0.46	2.16 \pm 0.11	5.41 \pm 0.11	64.92 \pm 0.56
V5	88.90 \pm 0.11	4.97 \pm 0.05	95.24 \pm 0.12	23.91 \pm 0.41	2.07 \pm 0.04	5.81 \pm 0.06	63.45 \pm 0.41
V6	88.04 \pm 0.04	5.47 \pm 0.01	93.82 \pm 0.04	22.65 \pm 0.25	1.91 \pm 0.05	5.75 \pm 0.15	63.52 \pm 0.09
P value	<0.001	<0.001	<0.001	<0.001	<0.01	<0.05	<0.001

Note: Values expressed in % of DM*

*Results are expressed as the mean ($n = 3$) \pm standard deviations (SD); DM = dry matter; CA = crude ash; OM = organic matter; CP = crude protein; EE = crude fat; CF = crude fibre; NFES = nitrogen free extractive substances.

Table 9. Chemical composition of the dry matter of runner bean seeds (%) (Data from the scientific literature)

CA	CP	EE	CF	NFES
3.5a	20.0a	1.5 ^a	3.1-4.4 ^b	51.99-54.71 ^b
3.3-4.2 ^b	21.64-26.69 ^b	1.3-1.9 ^b	4.02 ^c	30.1 ^c
4.1c	52.6c	7.53 ^c	10.5 ^d	53.1 ^d
4.6d	20.2d	7.9 ^d		

Note: CA = crude ash; CP = crude protein; EE = crude fat; CF = crude fibre; NFES = nitrogen free extractive substances; Different letters indicate the references: a = Kay, 1979, quoted by Popa (2010); b = Ivanov, 1961, quoted by Popa (2010); c = Aremu et al. (2006); d = Aremu et al. (2010).

L.) (2.1-4.1%), pigeon pea (*Cajanus cajan* L.) (3.8%) and African yambean (*Sphenostylis stenocarpa*) (3.5%). Our results are consistent with those reported by Ivanov (1961) quoted by Borcean and Borcean (2003) from Popa (2010), Kay (1979) quoted by Popa (2010), Aremu et al. (2006), Aremu et al. (2010), where crude ash ranged between 3.5 and 4.2%, crude protein 20.0-52.6%, crude fat 1.3-7.53%, crude fibre 3.1-10.5% and nitrogen free extractive substances 30.1-54.71% (Table 9).

Conclusions

The trellis system and intercropping system have an important influence on the runner bean plant phenology, assimilating pigments content in the runner bean leaves, agro productivity of runner bean crop, yield of runner bean crop and chemical composition of runner bean dry seeds (quality of the harvest). The best variant of trellis for runner bean crop was the single row trellis system on individual string and the best intercropping system was intercropping with sunflower. Runner bean crop significantly capitalizes the advantages of intercropping system, for example with maize and sunflower, ensuring a support system and a favourable microclimate for plant growth. Therefore, it is obviously that runner bean, in the ecological conditions of Romania, is a species that successfully can suit the intercropping system.

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