ROLE OF ARBUSCULAR MYCORRHIZA IN HEAVY METAL TOLERANCE IN SWEET BASIL PLANTS

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Abstract - The effects of phosphorus amendments and arbuscular mycorrhizal (AM) fungi Glomus intraradices on the sweet basil (Ocimum basilicum L.), chemical composition and percent of volatile oil, and metal accumulation in plants and its availability in soil were investigated in field experiment at two seasons under contaminated soil with Pb and Cu. The content of essential oil and shoot and root dry weights of sweet basil were increased by the application of mineral phosphorus as compared to control. Inoculation with AM fungi reduced the metal concentration in shoot, recording a lowest value of (33.24, 18.60 mg/kg) compared to the control (47.58, 23.70 mg/kg) for Pb and Cu, respectively. Availability of Pb and Cu in soil were decreased after cultivation in all treatments compared to control. However, metal root concentration increased with the inoculation, with highest values of (23.44, 39.25 mg/kg) compared to control (25.85, 34.34 mg/kg) for Pb and Cu, respectively. The content of linalool and methyl chavicol in basil oil was significantly increased in all treatments compared to control. We can thus conclude that the AM-sweet basil symbiosis could be employed as an approach to bioremediate polluted soils and enhance the yield and maintain the quality of volatile oil of sweet basil plants.

Index Terms - Arbuscular mycorrhizal fungus, heavy metals, sweet basil, oil composition

I. INTRODUCTION

Aromatic and medicinal plants are an important source of national income).The genus Ocimum comprises more than 150 species and is considered as one of the largest and foreign currency in Egypt. Basil is one of the most important species for export among the medicinal and aromatic plants and it has a good reputation in the European countries. . The area cultivated with basil in Egypt is about 4-5 thousand feddans, and the exports are more than 4000 tons per year [12] genera of the Lamiaceae family [13].

Ocimum basilicum L. (sweet basil) is an annual herb, plants is widely used in food and oral care products; the essential oil of the plant is also used as perfumery [5] and antimicrobial [8].

Environmental conditions and agricultural practices can significantly alter yield and chemical composition of sweet basil [34]. Monitoring for toxic heavy metals in medicinal plants has become part of the quality control in the pharmaceutical industry as consumers demand products that are free from potentially harmful constituents [9]. Phytoextraction potential of medicinal and aromatic plants grown in heavy metal polluted agricultural soils is a basil plants [28]. Arbuscular mycorrhizal fungi (AMF) symbiosis is formed by approximately 80% of the vascular plant species in all terrestrial biomass [35] which improves plant productivity (14). AM fungi can contribute plant growth, particularly in disturbed or metal polluted sites, by increasing plant access to relatively immobile minerals such as P, Zn, and Cu [32; 10]. In addition, they can improve soil structure [15], stabilize metals in the soil, reduce their uptake, and thus decrease the risk of toxicity to plants growing in polluted substrates [17].Thus, plants in symbiosis with AM fungi have the potential to take up heavy metals (HM).

The aim of the present study was to examine the potency of an isolate of AMF (Glomus intraradices), and to reduce metal accumulation in plant organs, and to evaluate how inoculation effect of mycorrhization and essential oil composition of (Ocimum basilicum L.) cultivated in Pb and Cu contaminated soil.

II. MATERIALS AND METHODS

Cultivated plant: Sweet Basil (Ocimum basilicum) seeds were obtained from Medicinal and Aromatic Plants Department, Agricultural Research Center, Ministry of Agriculture, Egypt. Seeds were germinated in seedling trail with 209 holes containing (1:1, v: v) peat moss and Sand. In case of mycorrhizal treatment (G3), the inoculant was applied to the nursery at the rate of 10% added to the mixture. Seeds were sown in the nursery on April 16th and the seedlings of 2-3 pairs of leaves were transplanted to the field at May 28th with one plant per hill.

Soil initial state analysis: Some physical and chemical properties of surface layer (0-30 cm) of the experimental field and heavy metals content (Pb and Cu) were determined according to [30] and [23]. The soil had the following physicochemical properties: clay loam in texture, pH (1:1 soil water suspension) 7.75 and 7.85, electrical conductivity (EC) 1.95 and 1.98 dSm^{-1},available P(15, 14.7 mg kg^{-1})soil,and the available Pb and Cu were (109,100) and (90.3, 88.6) mg/ kg for the two seasons, respectively.

Experimental design: The field experiment was conducted in Abees Experimental Farm, Faculty of Agriculture, Saba Basha, 10th Village, Alexandria University, Egypt during two seasons. The

experiment was carried out in a randomized split – split plot (10.5m^2, 3.5 x 3m) design in 3 replicates. Phosphorus fertilizer (0%, 75%, and 100% of the recommended dose) was the main plot, and the treatment un-inoculated was randomly distributed in the sub- plot, while the two treatments with Glomus intraradices (control un-inoculated and G3) were arranged in the sub – sub plots.

Inoculation of Arbuscular Mycorrhizal Fungus:
Mycorrhizal isolate of Glomus intraradices was used in this experiment; isolate (G3) was isolated from the Experimental Station of Alexandria University at Abees [1]. The inoculum consisted of expanded clay aggregates (2-4 mm in diameter, Leca) containing chlamydospores and fungus mycelium, which had been cullivated on Tagetes erecta L. [2]. Inoculant was added at the rate of 7.0 g/per hill below basil seedling after transplanted. Control plants received the same amount of heat sterilized expanded clay.

Mineral fertilizers: Three different rates (zero, 75% and 100%) of the recommended dose (400 kg/fed) of mono- calcium phosphate (15.5 % P_2O_5) was used in one dose at seedling transplanted for each growing seasons. Ammonium nitrate (33.5% N) was added at the recommended dose 150 kg/fed (50 kg N/ fed) after 30, 60, and 90 days of cultivation. Potassium sulfate (48% K_2O) was applied to all plots after 41 days of cultivation in the rate of 100 kg/fed.

The percentage of mycorrhizal colonization: The percentage of mycorrhization was estimated three times; (first, 4 weeks from germination to confirm the colonization before transfer to the field, second, 9 weeks old 1st cut and third at 16 weeks old 2nd cut). Root samples 1 cm were cleared with 10% KOH and stained with Trypan blue (0.05%) in lacto gelycerol to observe under microscope [24]. Mycorrhizal colonization was estimated by determining the percentage of length of root segments containing AM fungal structure (arbuscules, vesicles, spores) according to [6].
Root and shoot dry weights (g/plant): Five plants were air dried till constant weight and the average dry weight of root and shoot (g/plant) were calculated.

Chemical analysis
Phosphorus content: Plant powder (0.5g) was wet-digested with H_2SO_4 – H_2O_2 mixture [25). Phosphorus was determined by the Vanadomolybdate yellow method [21] using Millton Ray Spectronic 21 D.
Essential oil percentage: The essential oil percentage was determined in the air- dried herb according to [7] by water distillation of 40 g of air dry herb for 1.5 – 2.0 hours, in order to extract the essential oil.
Essential oil analysis and its major components: The essential oils were diluted in diethyl ether (20 :1,v:v) and analyzed with GC (HP 8644) with flame ionization detector (FID) on a fused silica 132 capillary column DB-5, 25 m in length, 0.32mm i.d., and 0.5 mm film thickness.133 Helium was used as the carrier gas with a flow rate of 1.6 ml/min; the detector 134 temperature was 260°C, the oven temperature was programmed to increase from 130 to 260°C at a rate of 4 °C/min. The split injector was heated at 250 °C; the split 136 ratio was 15:1. Data were processed on a DP 800 integrator. The percentage of major constituents of stander (β-caryophellene, Linalool and Methyl chavicol) was estimated by measuring the peak area of the different compounds of the chromatogram according to [18]. Standards of the principal components which used as reference compounds for sweet basil were obtained from Ciba Gigi, NY, USA.

Total lead and copper analysis: Heavy metals content in shoots and roots were determined by digesting the dried plant of each plot according to [25]. The disgusted solutions were analyzed for lead and copper using the atomic absorption spectrophotometer (A Analyst 400) according to [21]. Available lead and copper remain in soil after cultivation were measured by atomic absorption spectrophotometer model (A.A. Spectrometry Thermo Elemental Type Solar 54/2001, ser No. GE 710728) using diethyl enetriaminapenta acetic acid (DTPA) method as described by [30].

Statistical analysis: Data were statistically analyzed by the procedure of variance to test the treatments effect on different measured parameters according to [36]. Data for the percentage of root length colonization % were analyzed using angular transformation [37].

III. RESULTS

Mycorrhizal colonization percentage %
Mycorrhization was determined three times during every growing season before plant transplanting (4 weeks old) and two times after transplanting (9 and 16 weeks) the colonization at the 4 weeks was more than 33.3% that to confirm the colonization before transplanting. Root colonization by indigenous G. intraradices (G3) was low (Fig.1). Addition of P had no effect on mycorrhization in age 9 weeks but increased significantly after 16 weeks. However, the percent of root colonization was significantly higher in the basil inoculated with G. intraradices G3 than the non-inoculated plants.The AM root colonization in inoculated plants ranged from 39.00 to 50.00% and the native soil AM colonized roots were 7.70 % and 6.57 % at 9 and 16 weeks, respectively. Generally the mycorrhizal root colonization hadn't high value in this contaminated soil this could be attributed to the effect of heavy metals on the mycorrhizal spore germination, hyphal growth and branching.

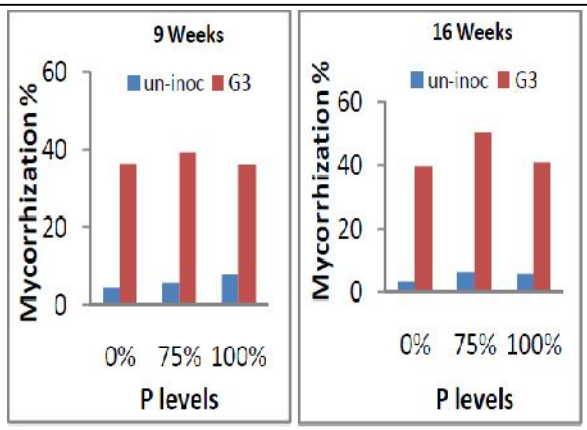


Fig. (1) Effect of inoculated Ocimum basilicum L. with Glomus intraradices on mycorrhization after 9 and 16 weeks

Shoot and root dry weight (g/plant)

Sweet basil shoot and root dry weights (Figures 2 and 3) responded significantly to phosphorus application and AM inoculation.

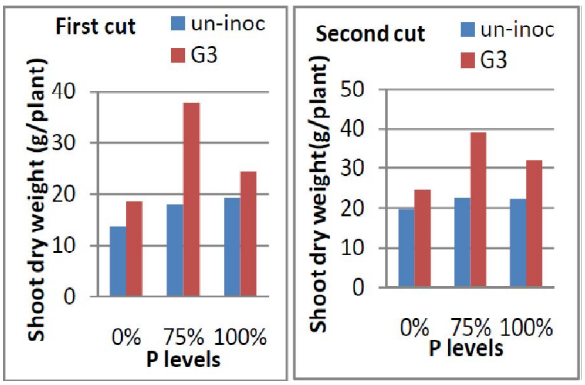


Fig. (2) Effect of inoculated Ocimum Basilicum L. with G. intraradices on shoot dry weight (g/ plant)

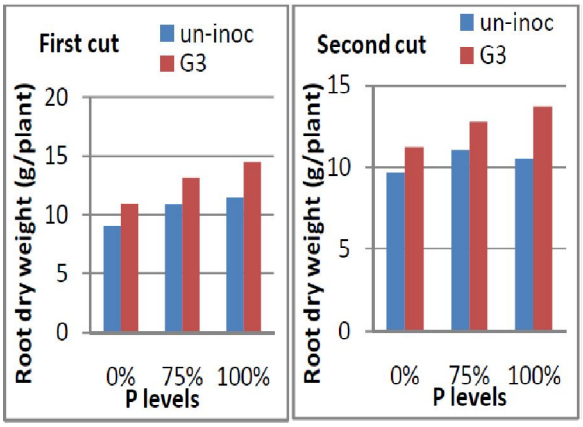


Fig. (3) Effect of inoculated Ocimum Basilicum L. with G. intraradices on root dry weight (g/plant)

Phosphorus uptake (mg/g shoot) and oil content

Data presented in Fig.(4) clearly show that the P uptake significantly increased. Application of phosphorus with different rates increased the phosphorus uptake of sweet basil plants, in the presence of AM inoculant. On the other hand the applied treatments improved the essential oil percentage compared with untreated plants Fig. (5).

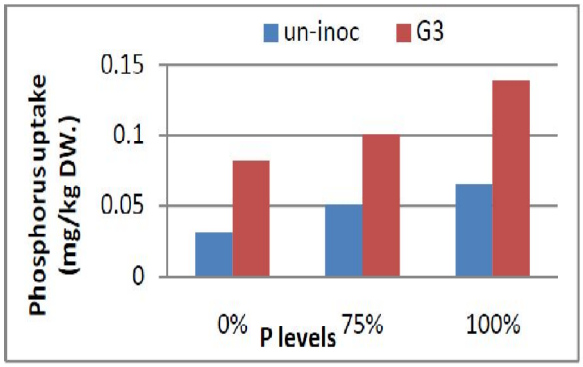


Fig. (4) Effect of inoculated Ocimum basilicum L. with G. intraradices on phosphorus uptake (mg/g shoot) in lead and copper contaminated soil as an avarage of the two growing seasons

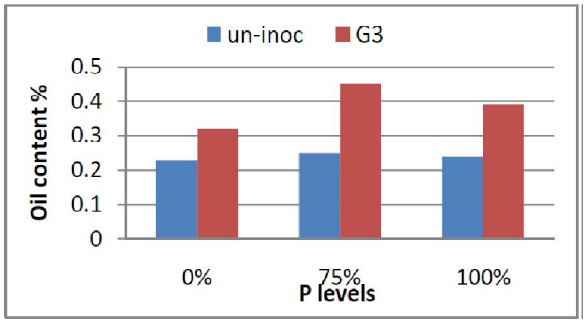


Fig. (5) Effect of inoculated Ocimum basilicum L. with G. intraradices on oil content % in lead and copper contaminated soil as an avarage of the two growing seasons

Fig. (5) showed that the percentage increase of oil content in plants inoculated with AM isolate G3 was (80%) ,more than the un-inoculated basil plants.

Major oil components (Linalool and Methyl chavicol %) of sweet basil plants

Chemical characterization of essential sweet basil oil (Figures 6 and 7) demonstrated that quality of oil improved on mycorrhization treatment in the presence of phosphorus fertilizer. Linalool the major component of sweet basil affected significantly with AM G3. Methyl Chavicol had the similar trend with inoculation; moreover the interaction showed no significant impact under contaminated soils. Data recorded in Figures (6 and 7) showed that the best results were obtained from G3 inoculation in the presence of 100% P, it were (52.61 % and 6.91 %) for linalool and methyl chavicol, respectively.

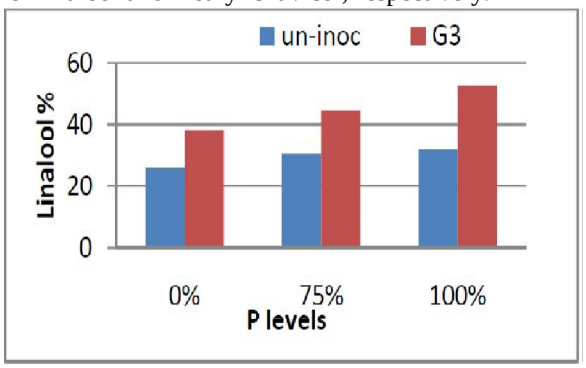


Fig. (6) Effect of Effect of inoculated Ocimum basilicum L. with G. intraradices on linalool % in the presence of different mineral phosphorus fertilizer rates as an avarage of the two growing seasons

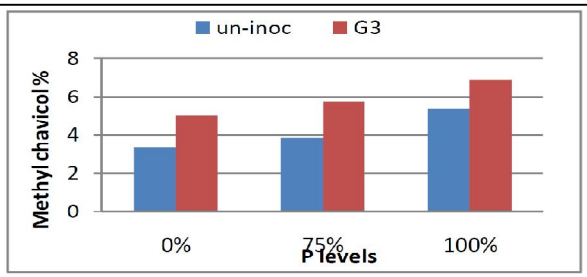


Fig. (7) Effect of inoculated Ocimum basilicum L. with G. intraradices on methyl chavicol % in the presence of different mineral phosphorus fertilizer rates as an avarage of the two growing seasons

Analysis of heavy metals

Lead concentration mg/kg in shoot , root content and residues in soil

Data presented in Fig. 8 "(a)" and "(b)" showed the effect of mycorrhizal inoculum in the presence of different phosphorus rates on Pb shoot and root content (mg/kg). Pb concentrations in sweet basil shoots, were highly significant affected by mycorrhizal inoculation. On the other hand Pb concentrations in shoots were increased by increasing phosphorus application and were decreased by the inoculation with AM isolate. Shoots of inoculated plants with G3 had the lowest value of Pb (33.24 mg/kg) showed in treatments with $P_{100\%}$. Opposite results were obtained in roots, lead concentration was higher in the inoculated plants than un-inoculated one. Low levels of Pb were measured in the control basil roots compared to treated plants. Significant reduction of Pb compared with the controls was observed in both soil and shoot of basil inoculated AM isolate. The same trend was observed in copper concentration, copper metal accumulation in shoot and a root tissue of sweet basil was significantly affected by application of AM inoculation Fig.9 "(a)" and "(b)". It was accumulated in very low concentration in shoot tissue (18.60 mg/kg) as compared to accumulation in control plants. Inoculation significantly increased the concentration of Cu in roots than shoots under all phosphorus fertilizer rates. However the highest value of soil available Pb (16.27 mg/kg) was observed in the absence of fungi and not amended with phosphorus fertilizer, whereas available Cu in soil reached (15.2 mg/kg). It is clear that the treatments reduce the soil available Pb and Cu compared to non-treated plants Figures (10 and 11).

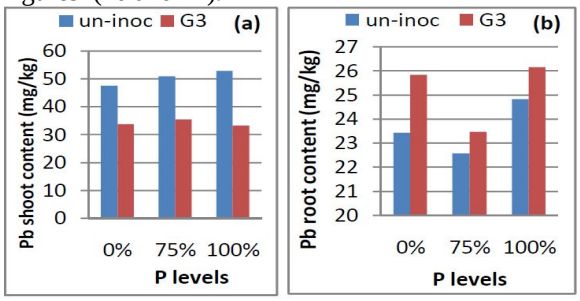


Fig. 8 "(a) and "(b)". Effect of Ocimum basilicum L. with G.intraradices on Pb content (mg/kg) in shoot and root in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons

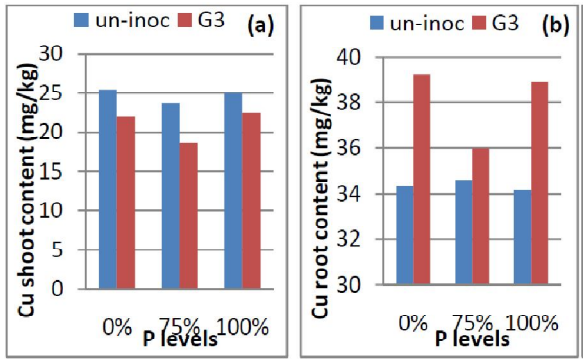


Fig. 9 "(a) and "(b)". Effect of Ocimum basilicum L. with G.intraradices on Cu content (mg/kg) in shoot and root in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons

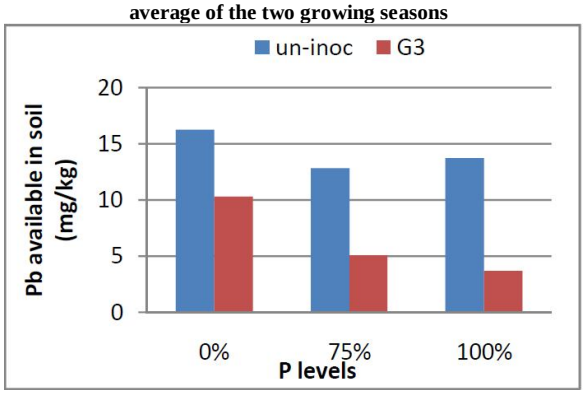


Fig. (10). Effect of inoculated Ocimum basilicum L.with G. intraradices on Pb available in soil after cultivation (mg/kg) in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons

Fig. (11). Effect of inoculated Ocimum basilicum L.with G. intraradices on Cu available in soil after cultivation (mg/kg) in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons

DISCUSSION

The present study demonstrates that colonization of basil plant root by the AM fungus G. intraradices was significantly affected by the application of different phosphorus fertilizer rates. Non-inoculated sweet basil plants showed >10.0% root colonization due to native mycorrhizae in soil and the percent of root colonization was significantly enhanced by plant inoculation with AM fungi under heavy metals (Pb and Cu) contaminated soil. Similar results were reported by [4] who observed that Glomus spp. treated plants had higher mycorrhizal colonization rates than other inoculation-treated plants. The data of the present work also showed that shoot and root of basil plant biomass increased in inoculated plant compared to control. Positive effects of mycorrhization on the growth of essential-oil-containing plants were reported with Ocimum basilicum L. [22] and Salvia officinalis L. [16]. The outcome of the plant-AM fungal association is metal-specific and depends on bioavailability of metals in soil and on both plant and AM species [38].

AM fungal inoculation increased shoot yield, content of essential oil, and root yield of sweet basil probably due to the increase of nutrient uptake [26]. Reference [4] reported that the two AM fungi species significantly increased the root and shoot dry weights and this species was more effective than non-mycorrhizal treatment in protection the maize plants against Cu toxicity. Reference [40] observed increased shoot and root growth weight, biomass and essential oil content of Ocimum spp. when inoculated with Glomus fasciculatum, Azotobacter chroococcum and A. awamori. Reference [19] found similar observations in Ocimum basilicum, where plant growth increased after inoculation with G. fasciculatum, Pseudomonas fluorescens and Bacillus megaterium.

Relatively little is known about the effects of AM colonization [20 and 22] on the accumulation of active phytochemicals in shoots of medicinal plants, which are often the harvest products. However, it was reported that Glomus mosseae directly increased the essential oil content in shoots in two of three tested oregano genotypes Origanum sp grown on industrially Cd and Pb polluted soil [22 and 20]. Similar results were obtained by [11] in studies with Ocimum basilicum L. Our results are in an agreement with the reports of [31] that the AM fungal inoculation maintained the level of linalool, methyl chavicol, in sweet basil oil, which were either increased or decreased by the application of heavy metals.

The arbuscular mycorrhiza (AM) fungi can increase plant uptake of nutrients especially relatively immobile elements such as P, Zn and Cu [32], and consequently, they increase root and shoot biomass and improve plant growth. It has been indicated that AM fungi can colonize plant roots in metal contaminated soil [40], while their effects on metal uptake by plants are conflicting. [41] reported that AMF increased lead accumulation in the roots. The most important finding of the present research is that AMF can keep metal concentrations in aromatic plants at low levels and alleviate harmful effects caused by heavy metal pollution. The inoculation with the AMF strains of EEZ 54 and EEZ 55, isolated from a place with naturally high levels of metals, reduced the Pb concentration in marjoram shoots and roots [20]. The effects of mycorrhizal colonization on remediation of contaminated soils depend on the plant–fungus–heavy metal combination and are influenced by soil chemical and physical conditions [33]. According to [3], AMF symbiosis may contribute to phytoremediation via strategies such as HM sequestration or accumulation, keeping metal concentrations in the plants below critical values and improving plant growth and nutrition [29]. Reference [31], demonstrated that AM species could be effective in protecting sweet basil exposed to high levels of metals. Mycorrhizal fungi are reported to protect plants from the toxic effects of high external concentration of several metals, possibly by binding the metals in their hyphae or by reducing the translocation of metals to the plants tops [27]. The higher AM-plant metal content of roots could be attributed to fungal metal binding and sequestration in intraradical hyphae, and these metal forms are not bioavailable to plants [10]. Plant under study accumulated more copper in roots but large reductions in shoots; this is in agreement with [4] that the comparisons of the two AM fungal species indicate that the AM fungal represented by Glomus spp (mixed) can benefit against potentially toxic Cu and therefore play a role in bioremediation of Cu-contaminated soils. Such results underline the importance of indigenous AM fungi, which are presumably more adapted to heavy metals.

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