

Original Research Article

Effect of different levels of mycorrhization on the growth parameters and nutrient content of chilli

Abstract:-

Aim: To evaluate the effect of different levels of mycorrhiza sp. (*Glomus mosseae*) on plant growth parameters chlorophyll content, mycorrhizal colonization(%), sporocarp number and nutrient content of chilli.

Study design: The experiment was conducted using a complete randomized design (CRD).

Study area:

To study the mycorrhizal effect on the chilli plants, observations were documented in Sreenhouse and laboratory conditions. Plant Pathology Laboratory, CCS HAU, Hisar was used for the laboratory work. The experiment was undertaken during crop season 2018

Methodology:

Mycorrhizal fungi was raised and maintained on wheat and pearl millet in earthen pots. Further experiment was conducted and Plant height, Root length, Dry weight of root and shoot, Mycorrhizal colonization, Sporocarp number, SPAD chlorophyll content and NPK content was observed.

Results:

Mycorrhiza is a symbiotic association between a fungus and the root system of vascular plants. Mycorrhizal associations help the host plants to thrive in adverse soil conditions and drought situations by increasing the root surface area and nutrient uptake efficiency. In the present study *Glomus mosseae* was tested on chilli plant with different inoculum levels (100, 150, 200 and 400 chlamydo spores/kg soil) and found that Plant height, Root length, Dry weight of root and shoot, SPAD chlorophyll content, per cent mycorrhizal colonization and sporocarp number were maximum when 400 spores were used for inoculation and minimum were found in untreated plants. Among all the four observation period i.e. 30, 45, 60 and 90 days after transplanting maximum NPK content was observed at 90 days after transplanting.

Conclusion: Among All the inoculum levels (100, 150, 200 and 400 chlamydo spores/kg soil) maximum plant growth parameters, NPK content and chlorophyll content was observed when 400 chlamydo spores/kg soil were used.

Keywords: Mycorrhiza, *Glomus mosseae*, days after transplanting, chlamydo spores, Inoculum

1. Introduction :

Mycorrhizal associations help the host plants to thrive in adverse soil conditions and drought situations by increasing the root surface area and nutrient uptake efficiency. AM fungi are obligate biotrophs, solely dependent on the host plants for their survival. Some bioactive molecules like strigolactones secreted by the roots help fungi to identify their host plants. Strigolactones also stimulate AM fungal growth and its branching. The arbuscular mycorrhizal fungi (AMF) found associated with the majority of land plants including

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those of the arid areas (1), once it established than it increases mineral nutrition uptake, mainly phosphorus and enhance plant growth. VAM not only increase the uptake of phosphorous, but also helps in uptake of zinc, copper, sulphur, potassium and calcium (2). Additionally, it protect plants against environmental stress such as soil salinity (3), drought (4) and control soil borne pathogens such as Fusarium wilt (5). In Kerala wilt infested area of solanaceous crops, the maximum mycorrhizal population was observed in Eruthyampathy of Palakkad district, the different mycorrhizal species (*Glomus* sp., *Acaulospora* sp. and *Sclerocystis* sp.) were identified. Arbuscular Mycorrhizal Fungi population was minimum in tomato and maximum in brinjal (6).

Phosphorus content of chilli plants was highest at 150% of organic manure application (7). Significantly more phosphorus content was observed in mycorrhizal plants than the non-mycorrhizal plants. It has been reported that plants inoculated with the *G. mosseae* has much thickened cell walls particularly at the edges as compare to uninoculated plants (8). Mycorrhiza is the most prevalent form of symbiosis relationship between plant roots and soil borne fungi. Since most of the economically important plant form mycorrhiza, the subject is gaining importance in agriculture, horticulture and forestry research. Arbuscular Mycorrhiza (AM) is well known for their plant growth promoting efficiency and providing bio protection against soil borne pathogens (bacteria, fungal and parasitic nematode). Therefore, an attempt has been made to see the effect of different doses of *Glomus mosseae* on growth parameters, sporocarp number, mycorrhizal colonization, mycorrhizal inoculation effect, mycorrhizal dependency and NPK content of chilli plants.

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Comment [a4]: Briefly introduce the chilli plant

2. Material and methods

The present study entitled "Effect of different levels of mycorrhization on the growth parameters and nutrient content of chilli" was executed in screen house and laboratories of Plant Pathology department CCS HAU, Hisar during 2018. The AM i.e. *Glomus mosseae* was taken from the department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar.

2.1. Maintenance of mycorrhizal fungi

In 20 cm diameter earthen pots the mycorrhizal fungi (*Glomus mosseae*) was maintained on wheat (*Triticum aestivum*) and pearl millet (*Pennisetum typhoides*). These pots were filled with 5 kg sterilized river sand. In upper 5 cm soil layer put one hundred g of mycorrhizal inoculum which contain about 450-500 chlamydospore and root bit and then ten seeds of wheat or pearl millet were sown and watered regularly. Hoagland's nutrient solution was applied @ 10 ml/pot after every 30 days of transplanting. After 90 days shoot portion of plant were cut at soil level and left the soil in pots to air dry. The soil was crumbled and cut the rootlets into 1 cm segments. This soil was used as a mycorrhizal inoculum.

Comment [a5]: Explain how to sterilize?

2.2. Mycorrhizal colonization

Mycorrhizal colonization was calculated by Staining of roots by following procedure (9).

2.3. Staining of root

Roots were cut into 1 cm segments, heat the roots in 10 per cent KOH at 90°C for one hour, washed these roots with fresh (10 per cent) KOH solution, immersed roots in alkaline hydrogen peroxide (H₂O₂) for 30 minutes. Then rinsed with distilled water to remove the excess of H₂O₂ and acidified with 5 N HCL for 30

Comment [a6]: Describe the method of inoculation of chilli plant with mycorrhizal inoculum and write the type of soil used to cultivate chilli

minutes. Roots were simmering in trypane blue in lactophenol (0.05%) for 5 min. Finally, roots were put in lactophenol to remove the extra dye and examine the roots under microscope.

$$\text{Mycorrhizal colonization (\% in roots)} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of sample assessed} \times \text{Maximum scale}}$$

Mycorrhizal dependency (MD):

Mycorrhizal Dependency (MD) is the degree to which a plant is dependent on the mycorrhizal condition in order to produce its maximum growth or yield at a given level of soil fertility. Mycorrhizal dependency was calculated by following formula given by (10).

$$\text{Mycorrhizal dependency (\%)} = \frac{\text{Dry weight of mycorrhizal plant}}{\text{Dry weight of non-mycorrhizal plant}} \times 100$$

Mycorrhizal Inoculation effect (MIE):

Mycorrhizal Inoculation Effect was estimated according to formula given by (11).

$$\text{Mycorrhizal Inoculation effect (\%)} = \frac{\text{Dry weight of mycorrhizal plant} - \text{Dry weight of non-mycorrhizal plant}}{\text{dry weight of mycorrhizal plant}} \times 100$$

Estimation of sporocarp in soil

Estimation of sporocarp in soil was done by Wet Sieving and Decantation Technique(12).

Estimation of Nitrogen content

For the estimation of nitrogen content, The Lindner method (1944) was adopted.

Estimation of phosphorous content

For the estimation of phosphorous content, Vanadomolybdophosphoric yellow color method (Koenig and Johnson, 1942) was adopted.

Estimation of potassium content

Potassium was determined in the acid digest of plant samples by using flame photometer (Elico CL 361, India) by direct reading.

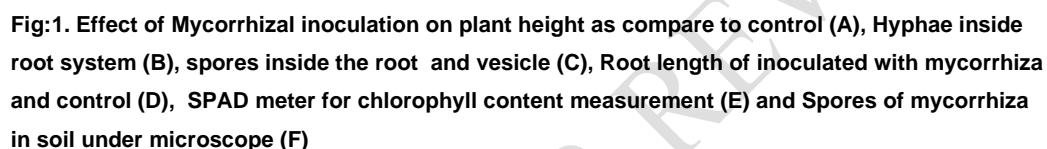
Chlorophyll content

Chlorophyll content of the plant was calculated by using SPAD chlorophyll meter.

3. Results and Discussion:-

The present study was done with the objective of best dose of mycorrhization in chilli plants. In general, AM fungal inoculation resulted in a general increase in plant growth parameters such as plant height, fruit yield, dry weight of shoot and root and plant P concentration as compared to the uninoculated plants. A pot experiment was conducted in screen house of Plant Pathology, CCS HAU, Hisar, to evaluate the effect of *Glomus mosseae* with different doses. Mycorrhizal colonization in roots and number of sporocarps in soil was calculated at 30, 45, 60 and 90 days after transplanting. Plant height, root length, dry weight of root and shoot, mycorrhizal inoculation effect, mycorrhizal dependency and SPAD chlorophyll content at 30, 45, 60 and 90 days after transplanting were recorded. Nutrient content (NPK) at 90 days after transplanting was calculated.

Comment [a7]: There is no need to write these content in the results and discussion



Plant height (cm)							
Inoculum levels (Sporocarp/Kg soil)	Days after transplanting (DAT)						
	30	45	60	90	Mean B	Mycorrhizal Inoculation effect % (MIE)	Mycorrhizal dependency % (MD)
100	10.53	12.03	18.17	24.37	16.28	54.98	222.11
150	12.43	14.53	19.27	26.50	18.18	62.70	268.07
200	13.67	17.97	21.40	28.93	20.49	66.86	301.75
400	15.87	19.53	24.37	32.47	23.06	69.65	329.47
C	9.57	11.43	14.93	19.77	13.93		
Mean A	12.41	15.10	19.63	26.41			
CD at 5 % level	DAT= 0.58 Inoculum level = 0.65 DAT×inoculum level = 1.30						

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Effect of soil application with different doses of AM fungi on plant height, Mycorrhizal inoculation effect and mycorrhizal dependency of chilli (Table 1). Application of the mycorrhizal species *Glomus mosseae* with different doses significantly increases in plant height of chilli at 30, 45, 60 and 90 days after transplanting. The maximum plant height (23.6 cm) was observed, when 400 chlamydospores/kg soil were used for inoculation followed by 200 chlamydospores/kg soil among the all the inoculum levels and minimum plant height was recorded in control (19.77). Irrespective of inoculum level maximum plant height was observed at 90 DAT. Maximum mycorrhizal dependency (329.47) and mycorrhizal inoculation effect (69.65%) was observed at 90 DAT, when 400 chlamydospores/kg soil were used the minimum mycorrhizal dependency (222.11 %) and mycorrhizal inoculation effect (54.98 %) was recorded when 100 chlamydospores/kg was used. Maximum plant height and growth parameters were found in inoculated plants and minimum was observed in uninoculated (control). The positive effect of mycorrhizal species was reported by (13) on all parameters like plant height, dry weight and root to shoot ratio increased, colonization of AMF, greatly increased chlorophyll content and root activity. Among the inoculum levels mycorrhizal fungi the maximum dry root weight was recorded in *Glomus mosseae* and minimum in control at 90 days after transplanting as compare to control. Dry biomass directly affects the mycorrhizal dependency and mycorrhizal inoculation effect. Similarly, (14) found that maximum dry shoot and root weight in mycorrhizal inoculated plants as compare to control. Fresh weight of shoot and root increased when AMF were inoculated (15).

Comment [a8]: Explain why the use of 400 chlamydospores/kg improved growth parameters?

Table: 2: Impact of soil application with different doses of AM fungi on root length and NPK content of chilli plant at 30, 45, 60 and 90 days after transplanting

Treatments	Root length (cm)					NPK content		
	Days after transplanting (DAT)							
	30	45	60	90	Mean B	N	P	K
100	8.03	9.20	9.90	10.08	9.303	1.16	0.41	1.67
150	8.83	9.84	11.90	12.13	10.675	1.18	0.43	1.77
200	9.27	12.49	13.10	13.23	12.023	1.19	0.50	1.81
400	10.17	14.13	14.93	15.53	13.692	1.21	0.60	1.89
C	7.23	8.40	9.20	9.93	8.692	1.12	0.38	1.43
Mean A	8.71	10.81	11.81	12.18				
CD at 5 % level	DAT = 0.27 Inoculum level = 0.30 DAT x inoculum level = 0.60					0.04	0.03	0.09

Comment [a9]: If you used statistical analysis, show it in the data table?

Roots length at different levels indicates that there was significant increase in root length,

by application of the mycorrhiza (*Glomus mosseae*) with different doses as compared to control. The maximum root length was observed, when 400 chlamydospores/kg soil was used among all the inoculum levels (100, 150, 200 and 400 sporocarp/kg soil) at 90 days after transplanting.

Table 3: Impact of soil application with different doses of AM fungi on Mycorrhizal colonization (%) and Sporocarp/ 200cc soil

Treatments	Mycorrhizal colonization (%)					Sporocarp/100g soil				
	Days after transplanting (DAT)					Days after transplanting (DAT)				
	30	45	60	90	Mean B	30	45	60	90	Mean B
100	32.33	43.00	57.67	71.00	51.00	25.67	39.33	55.67	67.67	47.08
150	37.67	47.00	68.00	75.67	57.08	27.00	48.00	62.33	74.33	52.92
200	40.67	57.67	76.00	81.33	63.92	36.67	62.67	75.33	85.33	65.00
400	46.67	63.33	81.33	87.00	69.58	39.67	80.00	97.33	120.33	84.33
Mean A	39.33	52.75	70.75	78.75		32.25	57.50	72.67	86.92	
CD at 5 % level	DAT = 1.85 Inoculum level = 1.85 DAT×inoculum level = 3.70					DAT = 2.07 Inoculum level = 2.07 DAT×inoculum level = 4.14				

In the study, percent mycorrhizal root colonization was recorded at 30, 45, 60 and 90 days after transplanting (Table 3). Irrespective of the days the maximum mycorrhizal per cent root colonization was observed, when 400 sporocarp/kg soil were used (69.58 per cent), followed by 200 sporocarp/kg soil were used (63.92 per cent), 150 sporocarp/kg soil were used (57.08 per cent) and minimum mycorrhizal per cent root colonization was observed 100 sporocarp/kg soil (51.00 per cent) were used. Among all the four observation period i.e. 30 DAT, 45 DAT, 60 DAT and 90 DAT, the maximum mycorrhizal per cent root colonization was observed at 90 DAT (78.75 per cent). Sporocarp number in soil was recorded at 30, 45, 60 and 90 days after transplanting. The maximum sporocarp number was found when plants were inoculated with 400 chlamydospores/kg soil. Irrespective of the days the maximum sporocarp number was observed, when 400 sporocarp were used (84.33) followed by 200 sporocarp (65.00), 150 sporocarp (52.92), 100 sporocarp (47.08). Among all the four observation period i.e. 30 DAT, 45 DAT, 60 DAT and 90 DAT, the maximum sporocarp number were observed at 90 DAT (86.92).

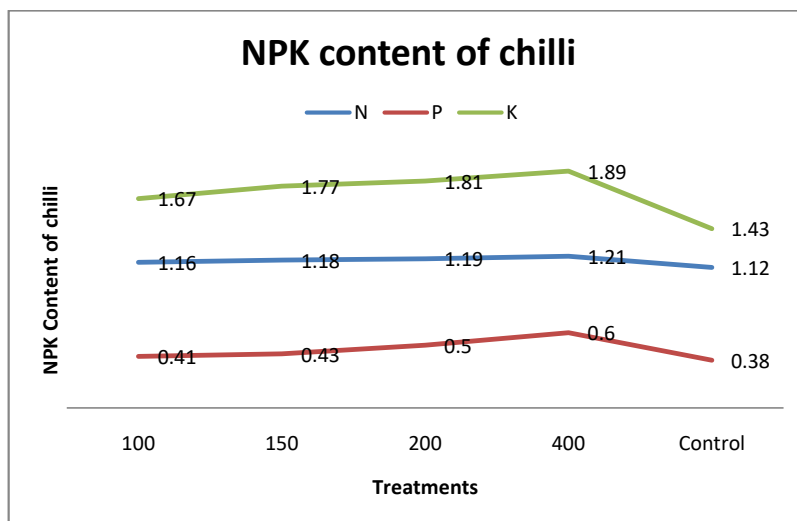


Fig 2: Effect of different doses of Mycorrhization on NPK content of chilli

The effect of mycorrhizal inoculation on NPK content of chilli was observed (Table 2 and fig 2). Maximum NPK content of chilli was found when 400 spores of *Glomus mosseae* were inoculated then 1.21 per cent N, 0.60 per cent P and 1.89 per cent K at 90 DAT was recorded and minimum NPK content was recorded in control (1.12 per cent N, 0.38 per cent P and 1.43 per cent K). The macro and micro nutrient contents *i.e.* N, P, K, S, Ca, Cu, Fe, Mn, Mg, and Zn was also improved in roots (13). Mycorrhiza fungi benefit the host plant by translocate phosphorus through a wide network of external hyphae and maximize the capability of the root system to absorb phosphorus. Nutrient content *i.e.* N, P, K, Fe and Zn uptake was also increased by VAM inoculation(16).

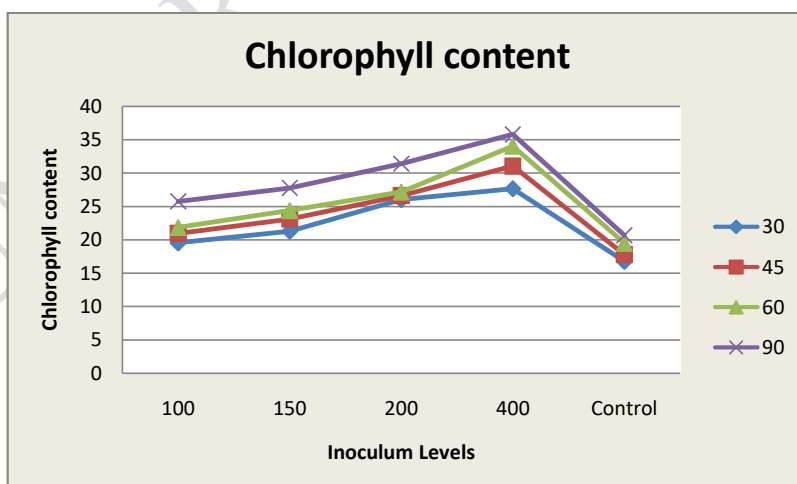


Fig:3. Impact of *Glomus mosseae* and their doses (100, 150, 200 and 400 spores/kg soil) on chlorophyll content of chilli

The application of the mycorrhiza (*Glomus mosseae*) with different doses (100, 150, 200 and 400 sporocarp/kg soil) significantly increase the chlorophyll content of chilli. Data was statistically analysed and found that the chlorophyll content varied significantly at 30, 45, 60 and 90 days after transplanting. The maximum chlorophyll content (Fig 3) was observed, when 400 spores were inoculated (32.42), followed by 200 spores (27.88), 150 spores (24.64), 100 spores (22.83) and minimum chlorophyll content was found in control (18.68). Positive effect of mycorrhizal colonization on the chlorophyll content of chilli was reported by many workers (17; 13; 18). Increase in chlorophyll content was also reported by (19) and (20).

Conclusion:-

The experiment was conducted to evaluate the effect of arbuscular mycorrhizal (*Glomus mosseae*) with different mycorrhizal inoculum levels (100, 150, 200 and 400 sporocarps/kg soil) on plant growth parameters, mycorrhizal per cent colonization and sporocarp number in soil, NPK content and chlorophyll content in the screen house of Plant Pathology, CCS HAU, Hisar. The maximum mycorrhizal colonization and sporocarp number, plant growth parameters (Plant height, Root length, Dry weight of root and shoot) and SPAD chlorophyll content was recorded, when 400 sporocarps/kg soil were used followed by 200 sporocarps/kg soil and minimum in control (Uninoculated).

References:

1. Stutz JC, Coperman R, Martin CA, Morton JB. Patterns of species composition and distribution of arbuscular mycorrhizal fungi in arid regions of southwestern North America and Namibia. *South African Journal of Botany*. 2000;78: 237-245.
2. Cooper KM, Tinker PB. Translocation and transfer of nutrients in vesicular arbuscular mycorrhizas. Uptake and translocation of phosphorus, zinc and sulphur. *New Phytologist*. 1978; 81:43-52.
3. Giri B, Kapoor R, Mukerji K. G. Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass and mineral nutrition of *Acacia auriculiformis*. *Biology and Fertility of Soils*. 2003;38: 170-175.
4. Al-Karaki GN, McMichael B, Zak J. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza*. 2004;14:263-269.
5. Habte M, Zhang YC, Schmitt DP. Effectiveness of *Glomus* species in protecting white clover against nematode damage. *Canadian Journal of Botany*. 1999;77:135-139.
6. Gopal S, Otta S K, Kumar S, Karunasaga I, Nishibuchi M, Karunasagar I. The occurrence of *Vibrio* species in tropical shrimp culture environments; implications for food safety. *International Journal of Food Microbiology*. 2005;102(2):151-9.
7. Dai O, Singh RK, Namasow G. Effect of arbuscular mycorrhizal (AM) inoculation on growth of Chili

Comment [a10]: You did not discuss at all in the results and discussion section, you only wrote the results

plant in organic manure amended soil. African journal of microbiology research.2011;5(28): 5004-5012.

8. Oyetunji OJ, Salami AO. Study on the control of Fusarium wilt in the stems of mycorrhizal and Trichoderma inoculated pepper (*Capsicum annum* L.). Journal of Applied Biosciences.2011;45: 3071– 3080.
9. Phillips J M, Hayman DS. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions British Mycological Society.1970;55: 158-161.
10. Plenchette C, Fortin JA, Furlan V. Growth response of several plant species to mycorrhizae in a soil of moderate P fertility. I. Mycorrhizal dependency under field conditions. Plant Soil.1983;70: 199- 209.
11. Bagyaraj D J. Vesicular-arbuscular mycorrhiza: Application in Agriculture. In: Norris JR, Read DJ, Varma AK (eds) Techniques for the study of mycorrhiza. Academic Press, London, pp. 1994:819-833.
12. Gerdemann JW, Nicolson TH. Spore density of *Endogone* species extracted from soil wet sieving and decanting. Transactions British Mycological Society.1963;46:235-244.
13. Chen S, Zhao H, Zou C, Li Y, Chen Y, Wang Z, Jiang Y. et al. Combined Inoculation with multiple arbuscular mycorrhizal fungi improves growth, nutrient uptake and photosynthesis in cucumber seedlings. Frontiers Microbiology.2017;8:1-11.
14. Sarwade PP, Chandanshive SS, Kanade MBMGA, Bhale UN. Growth effect of *Capsicum annum* var. Jawala plants inoculated with *Glomus fasciculatum* and *Trichoderma* species. International Multidisciplinary Research Journal.2017;1(12):13-16.
15. Matsubara VI, Haraba T, Yakuwa T. Effect of vesicular-arbuscular mycorrhizal fungi inoculation on seedling growth in several species of vegetable crops. Journal of Japanese Society for Horticulture Science.1994;63(3): 619-628.
16. Ali MM, Sani MdNH, Arifunnahar, Aminuzzaman FM, Mridha MAU. Influence of Arbuscular mycorrhizal fungi on growth, nutrient uptake and disease suppression of some selected vegetable crops. Azarian Journal of Agriculture 2018;5(6): 190-196.
17. Chaiya L, Kumla J, Suwannarach N, Kiatsirirot T, Lumyong S. Isolation, characterization, and efficacy of actinobacteria associated with arbuscular mycorrhizal spores in promoting plant growth of chili (*Capsicum flutescens* L.). Microorganisms. 2021;9(6): 1274.
18. Selvakumar G, Thamizhiniyan P. The effect of the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* on the growth and yield of chilli (*Capsicum annum* L.) under salinity stress. World Appl Sci J. 2011;14(8): 1209-1214.
19. Richmond, Amos E, Anton Lang. Effect of kinetin on protein content and survival of detached Xanthium leaves. Science. 1957;125: 3249 650-651.
20. Adivappar N. Effect of VAM fungi on growth, yield and drought tolerance of papaya M.Sc. (Hort)

Thesis Dharwad, India: University of Agricultural Sciences. 2001.

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