RESEARCH PAPER

Distribution of Arbuscular Mycorrhizae Fungi in the Rhizosphere of *Dodonaea viscosa* L. from a Semi-Arid Region

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ABSTRACT

In natural habitats, arbuscular mycorrhizal fungi (AMF) are essential for improving plant growth, nutrient uptake, and stress tolerance. The efficiency of various native fungal species across a variety of host plants in semi-arid climatic circumstances is still insufficiently known, despite having discovered that several studies demonstrate that AMF improves plant drought resistance. In the rhizosphere of *Dodonaea viscosa* L. in a semi-arid area, this study examined the variety of AMF, spore density, and root colonization. The conventional wet-sieving and decanting procedure was used to isolate AMF spores, and a standard root staining approach was used to measure root colonization. All study locations had consistently low electrical conductivity and low organic carbon content, according to soil physicochemical examination, indicating comparatively homogeneous soil fertility. Despite these circumstances, AMF root colonization was substantial in all studied locations (86.6–93.3%), indicating a robust and well-established mycorrhizal relationship with *D. viscosa*. *Rhizophagus sp.*, *Funneliformis mosseae*, and *Acaulospora scrobiculata* were the most prevalent AMF species in the rhizosphere. These results show how native AMF populations can adapt to semi-arid settings, proving ecologically significant and indicates their potential for the development of bioinoculants aimed to increase crop resilience and production under water scarcity conditions.

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INTRODUCTION

At the dawn of the twenty- first century, novel approaches to studying plants prompted a more detailed investigation of plant biodiversity, ecological connections, and microbial communities associated with them (Pagano et al. 2016). The mechanisms underlying microbial community recovery driven by Arbuscular mycorrhizae fungi (AMF) and aboveground plant diversity remain poorly understood, despite the widespread recognition of the ecological connection between aboveground and belowground biota. This represents a significant research gap in restoration ecology (Wang et al. 2021). The existence of bio tropic, root-associated mycorrhizae symbioses is an important evolutionary change that made it easier for plants to successfully colonize terrestrial habitats. In addition to their basic function in nutrient uptake, mycorrhizal relationships improve cellular water relations, increase resistance to environmental stressors, and help suppress pathogenic infections (Bellgard & Williams, 2011).

Soil microorganisms, especially those found in the rhizosphere, provide plants a sustainable way to Improve their health and increase their resistance to environmental challenges. One of these microbes, AMF, colonizes root cells to create a mutually beneficial symbiotic associations with plant roots. This symbiotic interactions is prehistoric and universal. Additionally, AMF enhances ecosystem processes and soil biodiversity, promoting plant growth and environmental sustainability (Boyno et al., 2025). Among the many symbiotic relationships that terrestrial plants have with microorganisms, AMF are among the most common and significant partners. This association gives rise to a specialized structure that facilitates the bidirectional exchange of nutrients between the plant and the fungus (Djezou et al. 2026). In recent years, increasing numbers of studies have examined how the composition of AMF communities varies under various environmental circumstances. AMF are essential elements of terrestrial ecosystems and play a major role in ecosystem function. However, there is still a lack of information regarding the species diversity and community structure of AMF in semi-arid regions (Zhao et al., 2017).

The potential of mycorrhizae symbiosis with plants is considered one of the most vital and enduring evolutionary adaptations to terrestrial environments. In a variety of plant species, these mutualistic associations have been extensively studied and recorded (Kumar et al., 2022). The ancient and geographical distributions of spore populations and AMF colonisation are determined by edaphoclimatic variables. AMF spore abundance, which typically reduces with increasing soil depth, is primarily affected by soil moisture, pH, and soil depth. Future studies should investigate the functions of various AMF species in distinct plant communities and dry habitats under varying climatic circumstances. To evaluate AMF reactions to heat and drought, long-term research is required. The use of organic amendments and bioinoculants, as well as more study on AMF interactions with other soil microbes, may assist in enhancing soil health and advancing sustainable agriculture in dry areas (Aloud et al., 2025).

Dodonaea viscosa is a common shrub species used to restore vegetation. Its enormous ecological flexibility is demonstrated by its worldwide distribution and capacity to flourish in a variety of climatic and ecological settings (Wang et al., 2018). Despite *D. viscosa* ecological significance, insufficient information is known about its rhizosphere-associated AMF in the semi-arid areas of southern India. This study investigates the diversity of AMF, spore density and root colonization in the rhizosphere of *D. viscosa* collected from Pudukottai District, Tamil Nadu, India, as well as their associations with certain edaphic variables. In addition to providing a basis for ecological understanding and future studies on the creation of native AMF inoculants to focus on heat-tolerant AMF exploration for crop improvement in semi-arid conditions, the results may help to promote plant establishment and sustainable restoration in semi-arid regions.

MATERIALS AND METHODS

Rhizosphere soil collection

Soil samples were taken from the *Dodonea viscosa* L. root zone. At a depth of 20 cm, approximately 500 g of soil and tertiary roots were removed. Samples were collected during May from three locations in the Pudukkottai district: Site 1 (Illuppur) lat 10.53 long 78.62, Site 2 (Avvor) lat 10.65 long 78.65, and Site 3 (Alangudi) lat 10.63 long 78.67. Each location had three replicate samples, which were then combined to create a composite sample. AMF spore isolation was carried out, the obtained samples were brought to the lab in sterile zip-lock bags and kept at 4ºC for further analysis; fine roots were stored in either formaldehyde or ethanol solution after being meticulously cleaned to rid of rhizosheath particles that were adhering to them.

Soil physiochemical properties

The feel method (Thein, 1979) was used to determine the texture of the soil. Calibrated pH and EC meters were used to test the electrical conductivity (EC) and pH of soil in a 1:1 soil-to-distilled water solution. The fast acid neutralization technique was used to measure the calcium carbonate concentration (Piper, 1966). The Olsen extraction method (Olsen, 1954) was used to measure available phosphorus (P), and the method described in Walkley & Black (1934) was used to quantify soil organic carbon (OC). The method outlined by Sankaram (1966) was used to evaluate the available potassium (K) and nitrogen (N). Additionally, the approach suggested by Lindsey & Norwell (1978) was used to evaluate the accessibility of micronutrients, including sulfur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and boron (B) (Table 1).

AMF spores isolation and identification

AMF spores were extracted from 100g of rhizosphere soil using a wet sieve and decanting method (Gerdeman & Nicolson, 1963). To ensure an equal dispersion of soil particles, the soil sample was subsequently mixed with 500 ml of distilled water and thoroughly stirred with a glass rod. Afterwards, the mixture was allowed to stand for a little while to the heavier particles to settle. A series of sieves with pore diameters of 720, 425, 250, 150, 75, and 45 µm were sequentially filled with the resultant suspension. Each sieve material was collected individually on Petri plates after being gently washed with distilled water. A light microscope (Magnus MLM) was used to measure the spore population at a magnification of 100x.

Number of spores per gram of soil = (Number of spores in 100 g of soil) / 100

PVLG alone or a 1:1 mixture of PVLG and Melzer reagent was placed on a glass slide after individual spores were carefully separated using a micropipette under microscopic direction. The preparations were sealed with DPX (dibutylphthalate polystyrene xylene) mounting media and coated with coverslips in preparation for further morphological investigation. PVLG-prepared slides were viewed at 40x magnification using an Olympus (CX31) binocular microscope fitted with an Optika digital camera. Based on diagnostic physical traits such as spore form, size, colour, wall features, and the kind of subtending hyphae, AMF spores were identified down to the genus level with the manual identification for AMF used by Schenk and Perez (1990). Using the current categorisation scheme found in the INVAM (http://invam.wvu.edu/collection), taxonomic identification and nomenclature were further confirmed.

Evaluate Root infection Percentage

The root cleaning and staining method established by Philips & Hayman (1970) was used to evaluate AMF root colonization. Fine root samples were carefully cleaned and divided into segments that were about 1cm long. While thicker or woody roots were sterilized in an autoclave at 121ºC and 15 psi for 30 minutes, the root pieces were cleaned in 10% potassium hydroxide (KOH) using a water bath kept at 90º C for an hour. The cleaned roots were quickly treated with alkaline hydrogen peroxide (H_2O_2) to remove any residual colours. After being acidified in 1% hydrochloric acid (HCl) for ten to fifteen minutes, the root segments were dyed with 0.05% trypan blue. To get rid of extra discolouration, the roots were submerged in lactoglycerol. The dyed root segments were then AMF colonization features such as hyphae, vesicles, and arbuscules were examined under a light microscope.

Mycorrhizal infection (%) = (Number of mycorrhizal root segments / Total number of root segments observed) ×100

RESULTS

Edaphic factors analyses

The physicochemical characteristics of the rhizosphere of *D. viscosa* varied slightly among the three sample locations (Table 1). Red sandy loam was found at site 3, whereas sandy loam was found at site 1 and 2. Every soil had low electrical conductivity (0.07-0.12 dS m^{-1}) and was not calcareous. While organic carbon stayed low (0.18-0.21 %), the pH of the soil varied from acidic (5.6 site 3) to alkaline (8.16, site 2). Phosphorus (10-12.5 mg kg^{-1}), potassium (51.5-71.5 mg kg^{-1}), and available nitrogen (8.5-10 mg kg^{-1}) slightly varied among the sites, with site 1 often having the greatest nutritional amounts. Micronutrients (Fe, Mn, Zn, Cu, and B) also showed very little variation, suggesting similar soil fertility throughout the sites of semi-arid region.

Spore density and root colonization

D. viscosa root colonization and AMF spore density varied significantly across the three sample locations (Figure A). Site 1 had the highest AMF root colonization (93.3 %) and spore density (2.15), followed by site 2 (1.84 spore density; 89.3 % colonization was consistently strong (>86%) across the *D. viscosa* rhizosphere study area, indicating a well-established AMF relationship despite the relatively huge spore quantities. Strong mycorrhizal connection to the rhizosphere of *D. viscosa* are observed in the semiarid area. The large number of live spores with full hyphal attachments may have maintained the high colonization levels seen in the *D. viscosa* rhizosphere, indicating the successful Establishment of AMF in a semi-arid environment.

Morphological identification of AMF

Using the conventional wet-sieving and decanting approach, AMF spores were effectively extracted from the rhizosphere of *D. viscosa*, producing an average 200 spores per 100 g of soil. Three different AMF morphotypes (Figure C) were found by morphological investigation; both had intact hyphal attachments, a sign of spore viability. All semi-arid sample locations showed high root colonization (86.6–93.3%; Figure A), indicating a robust and well-established mycorrhizal relationship. *Acaulospora scrobiculata*, *Funneliformis mosseae*, and *Rhizophagus sp*. spores were found in large quantities in the rhizosphere of the plant under investigation, indicating their extensive dispersion, according to morphological identification.

DISCUSSION

This study was carried out in May, during the season of high temperatures, because earlier research has shown that spore production is higher in summertime than in other seasons (Teixeira-Rios et al., 2018). Environmental factors, particularly warm-season temperature and frequency, are strongly associated with AM fungal colonisation levels in plant roots across ecosystems worldwide (Khan et al., 2017). These results indicates that the soil had low levels of organic carbon (OC) and electrical conductivity (EC), which is in line with earlier research defining these features. A decrease in soil organic matter is possibly linked to variations in the content of organic matter resulting from biological and chemical degradation processes (Bahadori & Tofighi, 2017). Microbial carbon consumption efficiency is influenced by temperature, although soil moisture and the makeup of the microbial population act as mediators. Usually, rising temperatures increase carbon losses via microbial biomass. In contrast, more effective microbial carbon use encourages microbial development and helps stabilise soil organic carbon (Brewer & Gaudin, 2020). AM provides major benefits in tropical soils that are acidic and low in nutrients (Prasad et al., 2017). Increased phosphorus levels probably precluded AMF colonization by reducing the plants' dependence on mycorrhizal symbiosis (Hebtemariam et al., 2026).

Using the conventional wet-sieving and decanting method, AMF spores were separated and identified from the rhizosphere of *Dodonaea viscosa*. There were three different AMF spore morphotypes, but there was an abundance of spores. The majority of isolated spores were intact and retained connected hyphae, indicating robust spore integrity. The present investigation's emphasis on morphological characterisation is consistent with earlier findings from semiarid habitats (Alguacil & Torres, 2016), which noted that spore morphology has traditionally been used for AMF identification. Despite advancements in molecular methods, this convention demonstrates the enduring use of morphological methodologies for ecological investigations. Mycorrhizal plants demonstrated enhanced resistance as well as improved growth and survival (Varma et al., 2017). The preservation of biodiverse habitats supports AMF, which are essential to plant development, ecological restoration, and maintaining the sustainability of natural ecosystems (Yelikbayev et al., 2020). A similar study conducted in dryland environments found high AMF colonisation rates (75–100%) across three tree species and three locations (Borde et al., 2009).

One of the most serious abiotic stressors that impacts plants worldwide is drought, and plants must be able to survive and flourish in arid conditions. While many plant species are more drought-tolerant when exposed to AM fungi, it is still unclear how effective different fungal strains are on diverse host plants in complicated environments (Guo et al., 2024). According to present research, *D. viscosa's* high degree of mycorrhizal colonization in its rhizosphere is probably related to its capacity to endure and adapt to semiarid settings, particularly in uncultivated natural habitats under drought stress. Similar to this, a morphological study of AMF linked to the Atlas pistachio tree (*Pistacia atlantica*) in Algeria's semi-arid and dry areas revealed root colonisation patterns Of both the Arum and Paris types. This discovery is consistent with current research and lends further credence to the idea that host plant traits influence AMF colonisation strategies and community composition in dry environments (Limane & Saadoun, 2022). Soil physicochemical characteristics, host plant species compatibility, and site-specific environmental variables are necessary to consider while choosing Mycorrhiza fungal strains to formulate as biofertilizers and bio protectants (Tripathi et al., 2017).

Further research is required to identify suitable native AM fungal strains associated with *Dodonaea viscosa* that might be effectively used to bio inoculants for crop improvements, restoration and sustainable management of natural ecosystems. Such studies would provide new opportunities for researchers and environmental conservationists to use Mycorrhiza as an environmentally sustainable tool for conserving, restoring, managing native AM fungal communities associated with *D. viscosa* and other tree species in dry land ecosystems.

Table 1. Edaphic factors analyses of *D. viscosa* rhizosphere from different sampling sites.

S. No	Edaphic factors	Site 1	Site 2	Site 3
1.	Soil types	Sandy loam	Sandy loam	Red sandy loam
2.	Calcareousness	Normal	Normal	Normal
3.	EC (dS^{-1})	0.07	0.12	0.11
4.	Ph	6.5	8.16	5.6
5.	OC (%)	0.18	0.21	0.18
6.	N (mg kg^{-1})	10	8.5	10
7.	P (mg kg^{-1})	12.5	10	10
8.	K (mg kg^{-1})	71.5	70	51.5
9.	S (mg kg^{-1})	4.165	6.2	6.2
10.	Fe (mg kg^{-1})	2.836	2.8	2.3
11.	Mn (mg kg^{-1})	2.821	2.85	2.85
12.	Zn (mg kg^{-1})	0.378	0.4	0.35
13.	Cu (mg kg^{-1})	0.436	0.4	0.4
14.	B (mg kg^{-1})	0.34	0.3	0.25

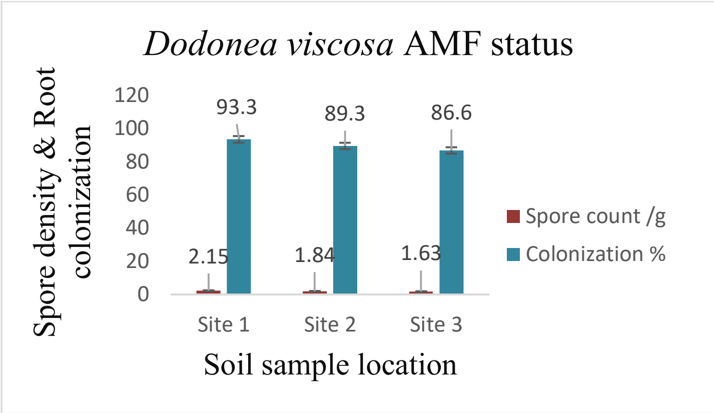

Figure A. Correlation between AMF spore density and root colonization of *D. viscosa* rhizosphere.

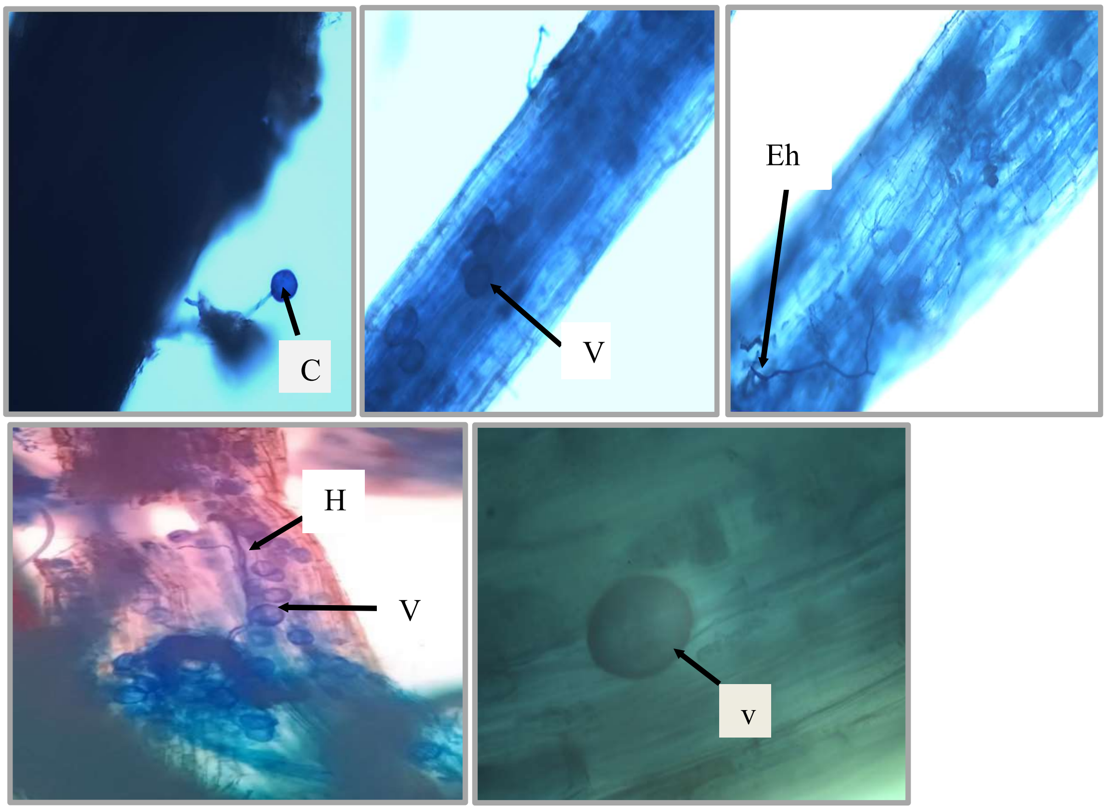

Figure B. Presence of mycorrhizal infection from *D. viscosa* rootlets shows hypha, vesicle, and spore (C-Chlamydospores, H- Hypha, Eh- Extraradical hyphae, V- Vesicle.

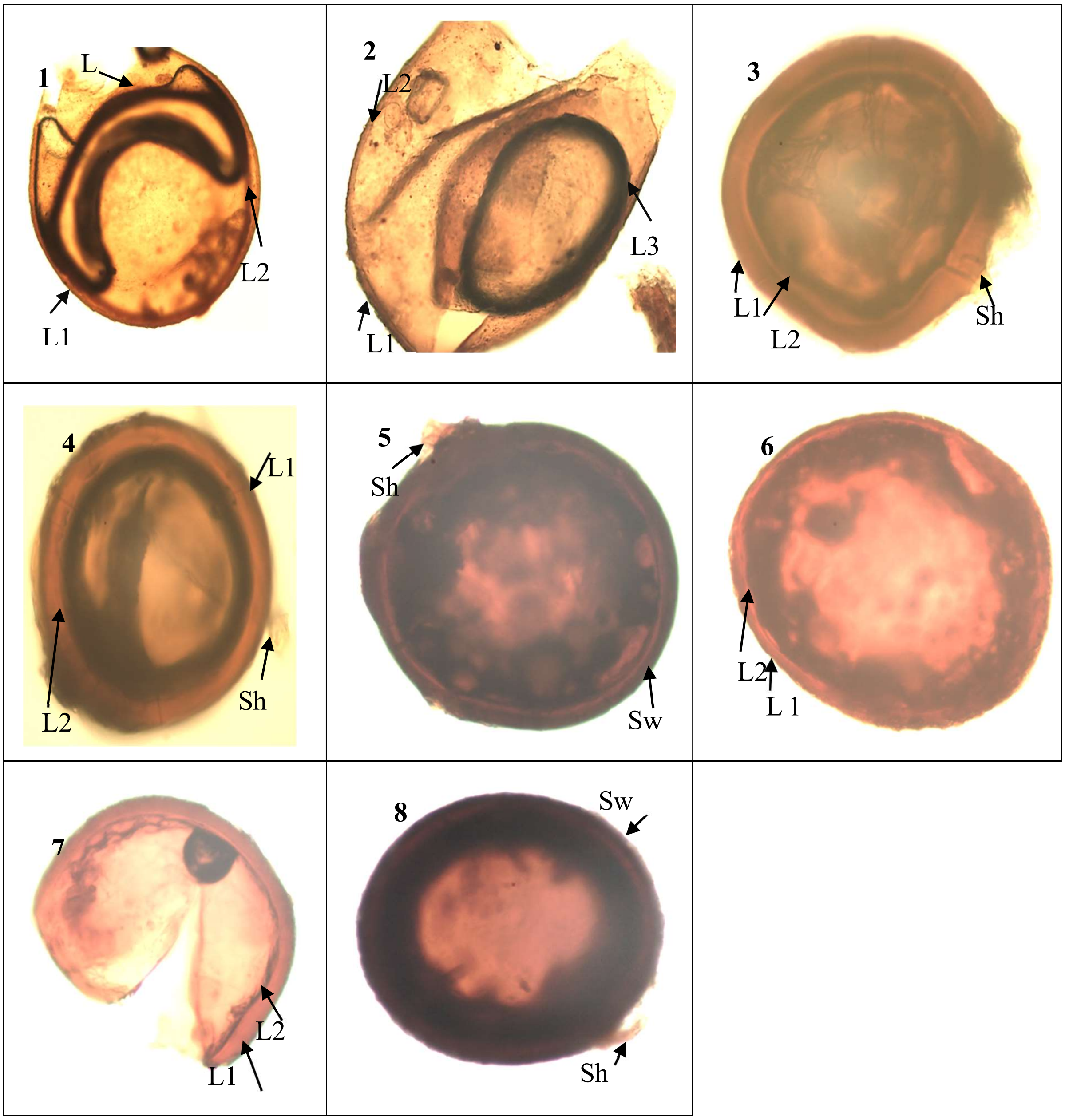

Figure C. Spore morphology of *D. viscosa* rhizosphere soil, L1- outer spore wall, L2- Inner spore wall, L3- germination shield, Sw- spore wall, Sh- Subtenting hyphae, 1,2- *Acaulospora scrobiculata,* 3, 4-*Funneliformis mosseae*, 5, 6, 7, 8- *Rhizophagus sp.,* (spore 6, 7 – light illumination state). (1, 2, 7- Crushed spores, 3, 4, 5, 6, 8-Intact spores).

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