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Diversity and Distribution of Arbuscular Mycorrhizal Fungi in Selected Medicinal Plants

L.S. Songachan[1], Anindita Deka[1], Subhesh Saurabh Jha[1]

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ABSTRACT

Background: Tha Arbuscular Mycorrhizal Fungi (AMF) association previously known as VAM belongs to an ancient fungal group known as Glomeromycota. In this study five commonly used medicinal plants viz., *Eclipta prostrata*, *Mentha arvensis*, *Elettaria cardamomum*, *Bacopa monneri* and *Mimosa pudica* were investigated for AMF colonization in the form of arbuscules, vesicles and hyphae from their roots and their rhizospheric soil was isolated for AMF spores. Majorly two genera of AMF dominated the rhizospheric soil of the plants investigated which were Acaulospora and Glomus. Altogether 12 AMF spores were isolated from the rhizospheric environment of the plants and they belonged to six different genera of AMF.

Methods: In this field-laboratory investigation during 2022-23, different localities of Banaras Hindu University was surveyed. 5 different plants were selected for investigation on the diversity and distribution of AMF colonisation found in their rhizospheric soil.

Result: Our investigation on the diversity and distribution of AMF of few selected plants from BHU have allowed us to isolate and morphologically identify 12 different species of AMF from 6 different genera. The geographical distribution of the collected species was discussed. The present work will be a complementary contribution to the comprehensive study of the identification and utility of AMF in plant growth and sust.

Key words: Arbuscular mycorrzhizal fungi (AMF), Colonization, Common mycorrhizal network (CMN), Glomalin.

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are a group of soil-dwelling organisms that form mutualistic symbiotic associations with the roots of the majority of land plants. These fungi belong to the phylum Glomeromycota and are known for their unique and intimate interactions with plant roots. Arbuscular mycorrhizal fungi play a crucial role in nutrient cycling and ecosystem functioning, as they facilitate the exchange of nutrients between plants and the soil (Brundrett *et al*., 1999; Li *et al*., 2006).

The AM fungal symbiosis begins when the fungal spores or hyphae come into contact with plant roots. The fungal hyphae penetrate the root tissues and establish a highly branched network called the arbuscule within the root cells. The arbuscule is characterized by highly branched structures that provide a large surface area for nutrient exchange. The fungal hyphae also extend into the surrounding soil, forming a vast network called the extraradical mycelium, which explores the soil for nutrients (Smith and Read, 2008).

The mutualistic nature of the AM fungal symbiosis is based on a mutual exchange of resources between the plant and the fungus. The plant provides the fungus with photosynthetically derived carbohydrates, which are transported through the plant's vascular system to the fungal structures within the roots. In return, the fungus enhances the plant's nutrient uptake, particularly for phosphorus (P), which is often limited in soil (Jha and Songachan, 2020). The fungal hyphae have a high affinity for phosphate ions, allowing them to scavenge and transport P to the plant. Additionally, AM fungi can also enhance the uptake of other nutrients such as nitrogen, potassium and micronutrients (Jha and Songachan, 2022).

[1]Department of Botany, Banaras Hindu University, Varanasi-221 005, Uttar Pradesh, India.

Corresponding Author: Subhesh Saurabh Jha, Department of Botany, Banaras Hindu University, Varanasi-221 005, Uttar Pradesh, India. Email: subheshjha2214@gmail.com

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The species richness of AM fungi can vary greatly across different habitats and ecosystems. Studies have revealed that AM fungal communities can consist of dozens to hundreds of different species. These fungi have been found to colonize the roots of a wide range of plant species, including both wild and cultivated plants, in diverse ecosystems such as grasslands, forests, wetlands and agricultural fields.

The diversity of AM fungi is influenced by various factors, including soil characteristics, plant diversity, land-use practices and environmental conditions. Soil properties such as pH, nutrient availability, organic matter content and moisture levels can shape the composition and diversity of AM fungal communities. For instance, certain AM fungal species may be more abundant in acidic soils, while others thrive in alkaline or neutral soils. The AMF richness and diversity also differ among different ecosystems and is usually greater in natural ecosystem compared to agricultural ecosystems (Wang *et al*., 2015).

Plant diversity plays a crucial role in shaping AM fungal diversity. Studies have shown that diverse plant communities support a higher diversity of AM fungi compared to monocultures (Mahanta *et al*., 2018). Different plant species exhibit varying degrees of specificity towards particular AM fungal species and the presence of diverse plant species can provide a wider range of niches and resources for AM fungi, leading to increased fungal diversity (Wang *et al*., 2018).

Land-use practices, such as agricultural practices and land management decisions, can also impact AM fungal diversity. Intensive agricultural practices, including the use of fertilizers, pesticides and tillage, can reduce AM fungal diversity by altering soil conditions and disrupting the symbiotic relationships between AM fungi and plants. Conversely, practices that promote sustainable agriculture, such as organic farming, crop rotation and the maintenance of plant diversity, can enhance AM fungal diversity by creating favorable conditions for their growth and establishment (Tchabi *et al*., 2008; Choudhary *et al*., 2009).

Environmental factors, such as climate and disturbance regimes, can influence the distribution and diversity of AM fungi. Different AM fungal species may exhibit varying tolerances to temperature, moisture and other environmental conditions. Changes in climate patterns, such as increased temperature or altered precipitation regimes, can potentially affect the composition and diversity of AM fungal communities (Fayuan *et al*., 2003). Additionally, disturbances such as fire, flooding, or land clearing can disrupt AM fungal communities, leading to changes in their diversity (Veresoglou *et al*., 2013; Xiang *et al*., 2014).

The identification of arbuscular mycorrhizal (AM) fungi can be challenging due to their complex life cycle and the absence of easily observable morphological features. Isolation and culture techniques involve extracting AM fungal spores or mycelium from soil or plant roots and establishing pure cultures (Landis *et al*., 2004; Gai *et al*., 2006). These cultures can then be subjected to morphological and molecular analyses for identification. Traditional morphological methods involve examining the structures of AM fungi under a microscope. The key morphological features used for identification include spore size, shape, color and wall ornamentation. Molecular methods, particularly DNA-based techniques, have revolutionized AM fungal identification by providing greater accuracy and resolving power. These methods involve the extraction of DNA from fungal samples, followed by amplification and sequencing of specific target regions, such as the small subunit (SSU) rRNA gene or the internal transcribed spacer (ITS) region. Phylogenetic analysis of the obtained sequences can then be used to identify the AM fungal species (Reddy *et al*., 2005).

It is worth mentioning that a combination of multiple methods is often used to achieve more reliable and comprehensive identification of AM fungi. The choice of method depends on factors such as the research objectives, available resources and the level of taxonomic resolution required. For this study we relied on morphological identification of AMF spores.

AMF undergo a complex life cycle characterized by a mutualistic association with plant roots. The life cycle begins with the germination of spores in the soil. These spores are typically dormant and resilient structures that can withstand harsh environmental conditions. Upon encountering a suitable host plant, the spores germinate and produce fine, branching hyphae. The hyphae then explore the soil, actively seeking out plant roots. Once a root is located, the hyphae penetrate the outer cell layers and establish a symbiotic association with the plant (Giovanneti *et al*., 1994; Hart and Reader, 2005).

MATERIALS AND METHODS

Study site and sampling

The study was conducted at BHU Campus, Varanasi. Two sites were selected for the current study namely botanical garden, department of botany (BG) and J.C. Bose hostel (JCB). The roots and rhizospheric soil of selected medicinal plants were collected from the above mentioned sites of BHU Campus. The plants selected for this study were *Eclipta prostrata*, *Mentha arvensis, Elettaria cardamomum, Bacopa monnieri, Mimosa pudica.* The sample were collected in sterilized plastic bags and transported to the laboratory for further analysis.

Estimation of AMF colonization

Root colonization was quantified using the technique of microscopic examination of root samples. Plant roots were carefully collected, washed to remove soil particles, cut into 1cm segments and then stained with trypan blue to visualize the fungal structures. Microscopic observation allows researchers to identify and measure the presence of AMF structures such as arbuscules, vesicles and hyphae within the root tissue. The percentage of root length colonized by AMF can be calculated based on these observations.

AMF spore isolation, identification and enumeration

AMF spores were isolated by technique known as wet sieving and decanting method. According to protocol, approximately 25 g of soil was taken and washed under running tap water through a series of sieves, the soil mixture was agitated vigorously to free the AMF spores from soil and allowed to settle for 15-45 minutes and the supernatant was decanted through standard sieves of 300 µm, 150 µm and 45 µm. The small particles left in the second tier (150 µm) were collected along with tap water and kept it in a beaker for 10-15 mins without any disturbance to settle down the debris below and spores on the surface. The water sample is then passed through a filter paper and the spores present along with debris were found on the paper and the water sample was then thrown. The filter paper was then spread on the petri dish and spores were counted using dissection microscope. Sporocarps and spore clusters were taken as single unit. AMF spores were picked up using a needle, mounted in PVLG. AMF spores were identified based on their morphological characteristics such as size, shape, wall ornamentation, color, etc. Spore density and species richness were expressed as number of AM fungal spores and numbers of AM fungal species in 25 g soil sample.

Soil physio-chemical analysis

Soil moisture was determined by drying 10g fresh soil at 105°C for 24 h in hot-air oven. Soil pH was determined using a digital pH meter. Soil physio-chemical properties of BG and JCB collected samples is given in Table 1.

Calculation and statistical analysis

For the purpose of this study various statistical parameters were used such as relative abundance, isolation frequency, Shannon-Wiener index of diversity, Simpson index of dominance, Evenness and Sorenson's coefficient of AMF (Dandan and Zhiwei, 2007). Spore density and species richness were expressed as the total number of spores and the number of species discovered in 25 g of soil. Pearson's correlation coefficient was used to examine the connections between AMF colonisation, spore density and soil physicochemical parameters. One-way ANOVA was used for statistical analysis and standard errors of means were determined.

RESULTS AND DISCUSSION

AMF colonization

Mycorrhizal colonization was higher in BG site than in JCB site. AMF structures, *i.e*. arbuscules, vesicles and hyphae and occasionally, intraradical spores were present in the root. Total AMF colonization ranged from 28.33% to 59.66% in BG site and 25% to 56.6% in the JCB site (Fig 2 and 3). In BG site, AMF colonization had a significant positive correlation with both moisture content ($r = 0.923$, $p<0.01$) and pH ($r = 0.828$, $p<0.03$). Spore density also showed positive correlation with moisture content ($r = 0.933$, $p<0.01$) and pH ($r = 0.958$, $p<0.01$). In case of JCB site, AMF colonization had a significant positive correlation with moisture content ($r = 0.913$, $p<0.01$) and pH ($r = 0.797$, $p<0.05$) and spore density also showed positive correlation with moisture content ($r = 0.919$, $p<0.01$) and pH ($r = 0.928$, $p<0.01$). ANOVA did not show any significant variation in mycorrhizal colonization among the two sites.

AMF species composition and diversity

Among the five investigated plants, AMF colonization was lowest in *E. prostrata* from both BG and JCB and highest in *M. arvensis*. It was found that in all plant species, colonization was highest in sample collected from BG compared to sample collected from JCB (Fig 1 and 2). Colonization in the form of vesicles was comparatively higher than arbuscules and hyphae in all plant species. AMF spore density is presented in Fig 3 and 4. It was lowest in *E. prostrata* (15 and 20 spores per 25gm soil in BG and JCB derived inoculum) and it was highest in *M. arvensis* (38 spores per 25gms of BG derived inoculum) in BG collected sample and *M. pudica* in JCB derived inoculum (52 spores per 25 gm of soil). Altogether 12 AMF spores were isolated from samples collected from BG and JCB (Fig 5). The 10 identified AMF species belonging to 6 different genera (4 species of *Acaulospora*, 3 of *Glomus*, 1 of *Funneliformis*, 1 of *Glomerulatum*, 1 of *Scutellospora* and 1 of *Rhizophagus*). Root colonisation structures of Arbuscules, hyphae and vesicles are shown in Fig 6.

All the diversity indices used to describe AMF communities are given in Table 2. AMF species isolated from the rhizospheric soil of selected plant from both BG and JCB derived inoculum with their relative abundance and frequency is given in Table 3.

P_i = Relative abundance of each identified species per sampling site and calculated by the following formula:

$$P_i = \frac{n_i}{N}$$

Where,

n_i = Spore number of a species.

N = Total number of identified species per sampling site.

Shannon-Wiener index of AMF diversity from sample collected from BG ranged from 1.45 to 2.25 while in JCB collected sample, it ranged from 1.32 to 2.0. Simpson's dominance index of AMF ranged from 0.15 to 0.16 from sample collected from BG, while it ranged from 0.17 to 0.24 in JCB collected sample. The value of evenness of AMF species ranged from 0.94 to 0.96 in BG collected sample while it ranged from 0.88 to 0.93 in JCB collected sample.

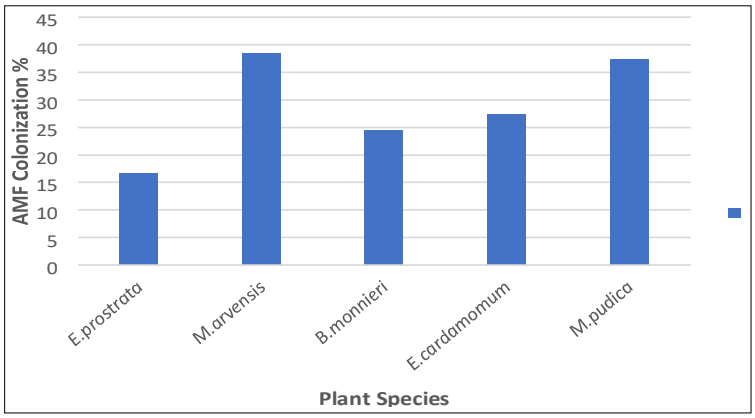


Fig 1: AMF colonization in the roots of different plants collected from botanical garden.

The plants selected for this study finds good applications in varied industries including pharmaceutical, herbal, agricultural, food as well as cosmetic industries. These plants have ethnobotanical values and been in use by people of Banaras region since time immemorial. Research on AMF has mostly revolved around the identification of AMF associations in different plant communities and their distribution patterns across different habitats in India. The result of this study showed that biodiversity of AMF differ from different plants and the extent of AMF is controlled by the host plants as well as environmental conditions. In our study, the diversity and distribution was described with the help of morphological features.

Table 1: Soil physio-chemical properties of BG and JCB collected sample.

Site	Moisture content (%)	Temperature (°C)	pH
BG	21±2.5	30±1.8	6.2±0.12
JCB	17± 2.2	32±1.9	6.4±0.13

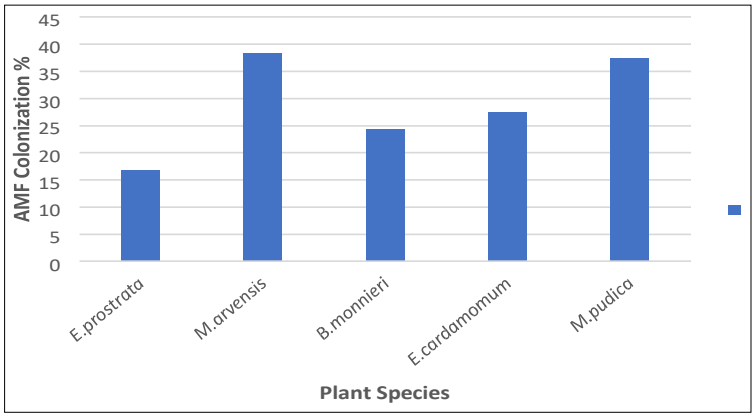


Fig 2: AMF colonization in the roots of different plants collected from JC Bose hostel.

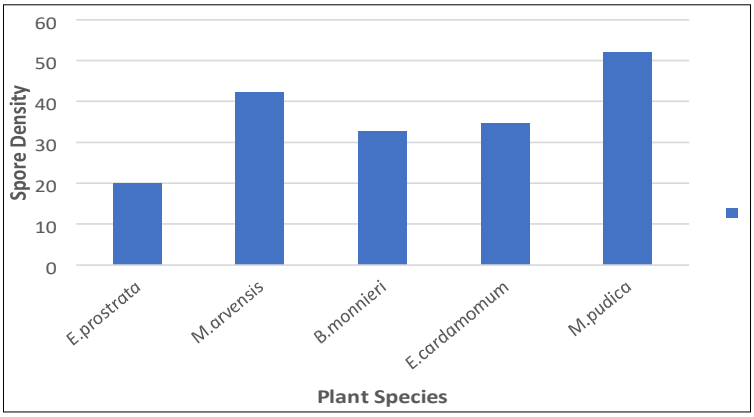


Fig 3: AMF spore density from the rhizospheric soil of different plants collected from Botanical garden.

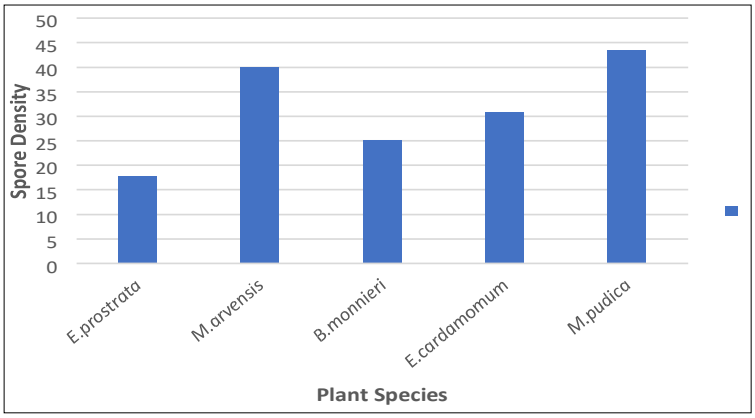
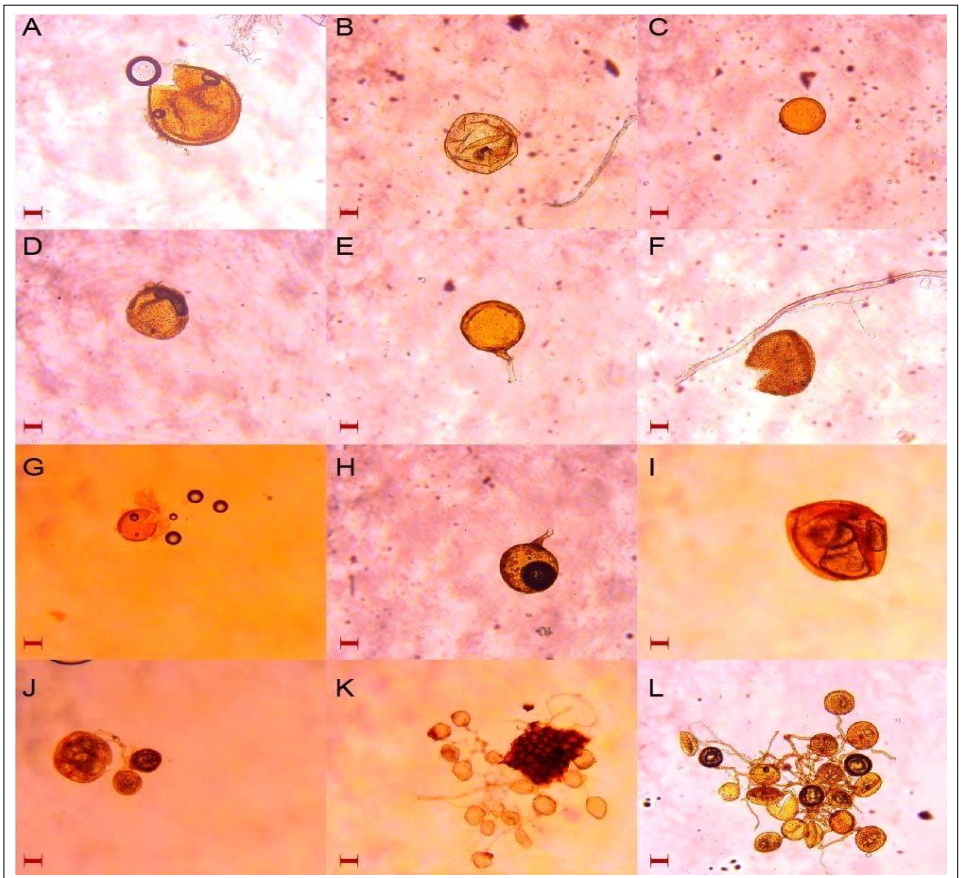


Fig 4: AMF spore density from the rhizospheric soil of different plants collected from JC Bose hostel.



(a) *Acaulospora koskei*, (b) *A. scrobiculata*, (c) *A. spinosa*, (d) *Acaulospora sps,* (e) *Funneliformis mossae*, (f) *Glomus glomerulatum*, (g) *G. macrocarpum*, (h) *G. maculosum* (i) *Scutellospora heterogama*, (J) *Rhizophagus aggregatus* (k) Sporocarp 1 (l) Sporocarp 2.

Fig 5: Isolated AMF species from the rhizosphere soils of selected medicinal plants.

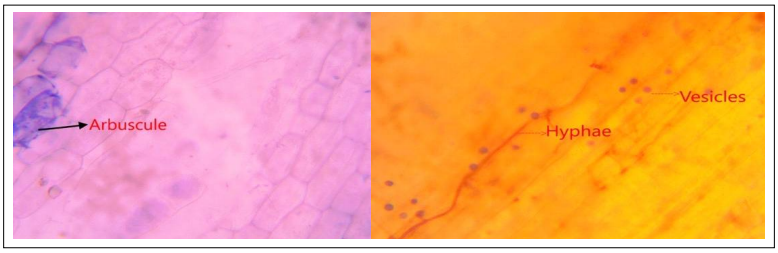


Fig 6: Root colonisation structures of Arbuscules, hyphae and vesicles.

Table 2: Diversity indices used to describe AM communities.

Spore density (SD)	The number of spores in 100 gm soil
Species richness (SR)	Number of identified AMF species per soil sample
Relative abundance (RA)	$\frac{\text{Spore number of a spices (genus)}}{\text{Total number of identified spore samples}} \times 100$
IF (Isolation frequency)	$\frac{\text{The number of soil samples in which AMF spices occured}}{\text{The total number of soil samples}} \times 100$
Simpson's index of dominance	$D = \Sigma (n_i/N)^2$
Shannon-wiener index of diversity (H′)	$H = \Sigma P_i \ln P_i$

Table 3: AMF species isolated from BG and JCB derived inoculum of selected plants.

AMF	RA% (BG)	RA% (JCB)	IF (%)
Acaulospora koskeii	5.55	4.25	100
Acaulospora scrobiculata	6.35	5.65	80
Acaulospora spinosa	2.25	3.15	100
Acaulospora sps	3.62	7.32	80
Funneliformis mossae	4.45	6.43	100
Glomus glomerulatum	2.02	3.55	20
Glomus maculosum	4.75	4.95	40
Scutellospora heterogama	4.24	6.27	20
Rhizophagus aggregatus	3.2	4.0	100
Sporocarp 2	3.2	4.6	80
Sporocarp 3	4.5	5.7	80

Note:- RA(%)= Relative abundance in percentage terms and IF(%)= Isolation frequency in percentage terms.

The result showed the dominancy of genus *Acaulospora* followed by *Glomus, i.e.* relatively produced more spores than *Funneliformis, Rhizophagus, Scutellospora* species in the same environmental condition. The degree of colonization and spore density varied markedly among plant species. Estimating AMF root colonization provides valuable insights into the extent and effectiveness of the symbiotic relationship between these fungi and plant roots. It enhances our understanding of the role of AMF in nutrient acquisition, plant growth and ecosystem functioning. Additionally, monitoring changes in root colonization over time or under different environmental conditions can provide insights into the dynamics of this important mutualistic relationship.

CONCLUSION

Arbuscular mycorrhizal fungi (AMF) has a pivotal role in modern agriculture due to their symbiotic association with plant roots, where they form structures such as vesicles, arbuscules, and hyphae within the root cells, as well as spores and hyphae in the rhizosphere. This intricate association between AMF and plants offers numerous benefits to the host plant, including enhanced nutrient uptake, improved stress tolerance, and overall growth promotion. This study revolves around the diversity and distribution of AMF in the rhizospheric environment of the of the selected plants. Further research will give us an insight of the ways AMF helps in growth and development of these plants.

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Conflict of interest

The authors declare that they have no conflict of interest.

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