Beneficial Endophytic Fungi of *Amaranthus* spp. An Interface Between Traditional Medicine and Modern Drug Discovery

Chandrama Mukherjee, Debapriya Choudhury, Shinjan Dey & Sikha Dutta*

Author details:

1. Chandrama Mukherjee
2. Debapriya Choudhury
3. Shinjan Dey
4. Sikha Dutta (Corresponding author)

Applied and Molecular Mycology and Plant Pathology Laboratory
Department of Botany, UGC-CAS, The University of Burdwan, Purba Bardhaman-713104

Abstract: In the internal tissues of plants lives a fascinating community of microorganisms known as endophytes. Endophytes have a beneficial, neutral, or pathogenic impact on their host plants. These tiny inhabitants play a crucial role in plant health, influencing growth, resilience, and defence mechanisms. Their presence depends on factors like, the plant species, growth stage, environmental conditions, and microbial density. Beyond their ecological role, endophytes are a treasure house of bioactive compounds i.e., phenolics, flavonoids, alkaloids, terpenoids, tannins etc. which have promising medical applications. *Amaranthus* belongs to the family Amaranthaceae. Endophytes work silently within plants and their interactions with plants like *Amaranthus* open doors to groundbreaking applications, from new medicines to

sustainable agriculture and environmental protection. Some new technologies i.e., Liquid Chromatography, Deep learning methods which are utilized for synthesizing novel medicine are discussed here. This review explores the hidden world of endophytes colonized within the plants with the natural richness of *Amaranthus* could help in synthesis of novel medicines and makes a bright future.

Keywords: *Amaranthus*, endophytes, new technologies, novel medicine, bioactive compound.

1.Introduction: An endophyte is an endosymbiotic group of organisms, often a bacteria or fungi that colonize in the intracellular or intercellular spaces of plants [1,2]. Endophytes may be beneficial symbionts, neutralists, commensals, or may be pathogenic. Endophytes can colonize in the internal tissues of plants, especially in the stem, roots, leaves, inflorescence, fruit, buds, seeds and also dead hollow hyaline cells of plants [3,4,5]. The distribution of endophytes in a plant species is not fixed and depends on various factors such as, host species, host developmental stages, environmental conditions and inoculum density [6]. The endophytes are great source of bioactive compounds i.e., phenolics, flavonoids, alkaloids, terpenoids, steroids etc. which have a huge importance in medicinal world and are capable of transforming natural products by changing their structure and activities [7]. Endophytes act as linking plant, rhizosphere microbes and the soil to boost up nutrient access and delivery to plant roots, creating the soil-plant-microbe continuum [8]. Endophytes secrete secondary metabolites that are believed to enhance host plant fitness and survival by providing protection against pathogens [9].

The genus *Amaranthus,* belonging to the Amaranthaceae family, encompasses a diverse group of plant species, many of which are distributed all over the World. *Amaranthus* mainly consists of 400 species, which are cultivated presently. But, the total number of species under this genus are need to be determined. The genus includes leafy vegetable amaranth, grain amaranth, ornamental and weedy Amaranth [10]. In Aztec, Mayan, and Incan civilizations, *Amaranthus* was a vital food source and also had religious significance. Additional important advantages of this plant are satisfactory yield performance, drought resistance, and enhanced photosynthesis. There has been a renewed interest in *Amaranthus* sp. as a nutraceutical and natural remedy for chronic diseases in recent years [11].

Amaranthus extracts have been traditionally used in Indian, Nepalese, Chinese, and Thai medicine to treat a wide range of conditions, including urinary tract infections, gynaecological disorders, diarrhoea, pain, respiratory issues, diabetes, and as a diuretic [12,13]. In India, the root extract of *A. spinosus* is used as a vermifuge by the Santali and Paharia tribes of eastern Bihar, while an aqueous decoction of the plant is administered for chronic diarrhoea in southern Orissa. Some tribes also use to induce abortion [14]. In Kerala, tribal communities use the plant's juice to reduce abdominal swelling, and its leaves—boiled without salt—are consumed over 2–3 days as a remedy to cure jaundice. In recent years, scientific interest in *Amaranthus* and its health-promoting benefits has grown considerably, with numerous reviews exploring its nutraceutical properties [15] applications and processing techniques gap. Occurrence of endophytic fungi associated with different species of Amaranths are shown in Table1. Pictures of different species of *Amaranthus* are diagrammed in Fig1.

In this review, we described the diverse medicinal properties of Amaranths, potential bioactive secondary metabolites are extracted from it and different mechanisms of endophytes

which protect the host plant from different biotic and abiotic stresses. The novel approaches for producing drugs or used in the treatment of chronic diseases are outlined here i.e., Deep learning methods, One strain many compound (OSMAC), Liquid chromatography (LC) etc.

Table 1: Fungal endophytes commonly associated with Amaranthus:

Sl. No.	**Genus**	**Endophytic fungi**
1.	*Amaranthus spinosus*	*Aspergillus* sp., *Phoma* sp., *Fusarium* sp., *Cladosporium sp*
2.	*A. viridis*	*Chaetomium globosum*
3.	*A. hybridus*	*Alternaria* sp., *Phoma* sp., *Fusarium* sp.
4.	*A. cruentus*	*Albifimbria* sp., *Alternaria* sp.
5.	*A. retroflexus*	*Alternaria sp., Cladosporium* sp., *Phoma* sp., *Penicillium* sp., *Fusarium* sp. *Geotricum* sp., *Botrytis* sp. *Trichoderma* sp.
6.	*A. hypochondriacus*	*Alternaria* sp., *Aspergillus* sp. *Penicillium* sp., *Phoma* sp. *Rhizopus* sp., *Trichoderma* sp.
7.	*A. tricolor*	*Alternaria* sp.
8.	*A. caudatus*	*Alternaria* sp.
9.	*A. blitum*	*Trichoderma* sp., *Penicillium* sp. *Aspergillus* sp.
10.	*A. dubius*	*Alternaria* sp., *Cladosporium* sp.

Data adapted from Pusz et al., 2015; Yang et al., 2023 [16,17]

Fig 1: a. *Amaranthus caudatus*, b. *A. cruentus*, c. *A. hybridus*, d. *A. tricolor*, *A. hypochondriacas*)

2.Medicinal properties:

In recent times, there has been a renewed interest in *Amaranthus* species for their potential as nutraceuticals and natural agents to resist chronic diseases [18]. The anti-cancer, antiviral, hepatoprotective, neuroprotective, cardioprotective, and antidiabetic properties of *Amaranthus* have come into sharp focus in recent times, owing to their relevance in addressing contemporary global health challenges. Natural crude extracts from plants have long been utilized in traditional medicine to address a variety of health conditions, with *Amaranthus* spp. being one such example [19]. Medicinal activities are represented in a flow chart Fig2.

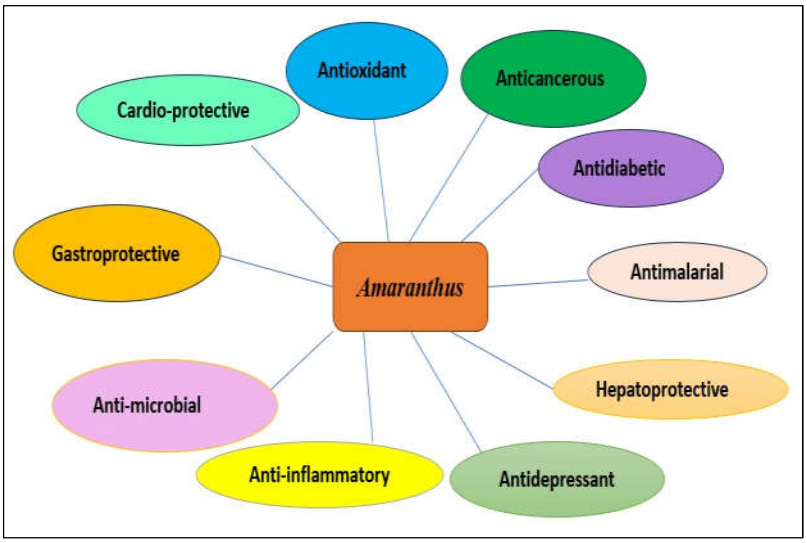

Fig2: Medicinal properties of *Amaranthus*

2.1. Antioxidant: Antioxidants are molecules that help reduce the damage caused by free radicals, playing an important role in protecting against cancer and degenerative diseases. *Amaranthus* spp. is rich in these antioxidants. The leaves and flowers of Amaranths, along with their extracts, have been found to show the highest antioxidant activity compared to other parts of the plant [20].

2.2. Hepatoprotective: Methanolic extract of *A. spinosus* increased protein and glycogen in livers of Sprague Dawley rat showing that it may be safe for treating liver problems [21]. Methanolic extract of the whole *A. spinosus* plant demonstrated marked hepatoprotective effects against paracetamol-induced liver damage in Wistar rat [22].

2.3. Antidiabetic properties: The methanolic leaf extracts of *A. caudatus, A. spinosus*, and *A. viridis* demonstrated significant hypoglycaemic and cholesterol-lowering effects and also have the property in treatment of hyperglycaemia and used as nutraceuticals of diabetes [23,24].

2.4. Anti cancerous: Water-based extracts of two amaranth species, *A. dubius* and *A. hybridus*, were tested for their anticancer effects. In female Swiss albino mice inoculated with Ehrlich's ascites carcinoma (EAC) cells, treatment with test extracts (25, 50, or 100 µg/mL/day/mouse) commenced 24 hours post-injection. The suppression of tumour cell growth was subsequently evaluated. Treatment with *A. hybridus* and *A. lividus* extracts resulted in 45% and 43% inhibition of EAC cell growth, respectively with augmentation of p53, Bax and caspase-3 and repression of Bcl-2 mRNA. *A. hybridus* seed extract showed stronger anticancer activity than *A. lividus* stem extract across all tested doses. Along with growth inhibition, amaranth extracts induced apoptotic morphology in EAC cells, highlighting their therapeutic potential for cancer research. Hexane, ethyl-acetate and Methanolic extracts of *A. tristis* demonstrated significant antiproliferative activity against COLO-320-DM human colon adenocarcinoma cells, with minimal observed cytotoxicity [24].

2.5. Anti-inflammatory: Extracts from *A. lividus* and *A. tricolor* exhibit anti-inflammatory effects by suppressing pro-inflammatory cytokine gene expression (Baraniak et al. 2022). The first description of anti-inflammatory bioactive peptides derived from germinated amaranth via in vitro gastrointestinal digestion reported in the scientific literature in 2021[25].

2.6. Gastroprotective: Many natural remedies made from plants have been used in clinical and folk medicine due to their beneficial effects on the mucosa of gastro- intestinal tract. A poly-herbal formulation comprising leaf extracts of *A. tricolor* along with two additional medicinal herbs demonstrated significant antiulcer activity. Ethanolic and ethyl acetate leaf extracts of *A. tricolor* exhibited ulcer-healing properties in rats with acetic acid-induced chronic gastric ulcers [26]. Powdered leaves of *A. spinosus* provided significant protection against ethanol-induced gastric ulcers and cysteamine-induced duodenal ulcers in Wistar albino rats. A different poly-herbal formulation, combining the hydroalcoholic extract of *A. roxburghianus* roots with piperine, demonstrated minimal ulceration, haemorrhage, necrosis, and leukocyte infiltration upon histopathological examination. Additionally, it reduced myeloperoxidase and MDA levels while enhancing glutathione concentrations in both blood and colon tissues of rats with ulcerative colitis [27].

2.7. Antimalarial: *A. hybridus* is among the plant species traditionally used by the Msambweni community on Kenya's South Coast (where the disease is endemic) for the treatment of malaria. In a study with animals, giving an ethanolic extract of *A. spinosus* to mice infected with *Plasmodium berghei* led to weight gain, higher haemoglobin levels, and strong anti-malarial effects, similar to those of chloroquine [28].

3.Bioactive metabolites from endophytic fungi in Amaranthus:

It has been reported to have several bioactive constituents like alkaloids, flavonoids, phenolics, steroids, terpenoids, saponins, betalains [29].

3.1. Phenolics: Phenolics reduce the risk of heart diseases and exhibit anti-inflammatory properties, primarily through their ability to scavenge free radicals [29]. Amaranth leaves were found to contain nine phenolic compounds, including coumeric, vallinic, caffeic, syringic, ferulic, sinapic, protocatechuic, salicylic, and 4-hydroxybenzoic acid [30].

3.2. Flavonoids: Flavonoid contains antioxidant agents, it reduces the oxidation of low-density lipoprotein (LDL), lower cholesterol level and triglyceride. It is also expressed antimicrobial property [29]. The main flavonoids found in the sprouts of *A. cruentus* was rutin, myrecetin, quercetin, vitexin, isovitexin and morin were also detected in the sprout and seeds. *A. blitum, A. caudatus* and *A. tricolor* species had aglycones in their roots. Aglycones were found in *A. albus* and *A. blitoides* species inflorescences. Kaempferol, rutin, myricetin and vitexin were found. Apigenin and morin also found [31].

3.3. Betalains: Recently have been recognized as highly bioactive natural compounds with potential human health benefits. Betaxanthins (yellow pigments) and betacyanins (amaranthin, isoamaranthin) are nitrogenous water-soluble vacuolar pigments used in folk medicine and also as anticancerous, anti-inflammatory and cardiovascular agents [32].

3.4. Alkaloids: Alkaloids play a vital role in the metabolism and development of living organisms. It is a beneficial metabolite which serves as resistant to parasites and predators and also have antimicrobial activity [29].

3.5. Terpenoids: Terpenoids are recognized for their diverse therapeutic properties including anticancerous, antiparasitic, antimicrobial, antifungal, anti-inflammatory, antiviral, antiallergic and antispasmodic effects [33].

3.6. Steroids: Steroids play a crucial role in pharmaceutical drug development because they contain compounds that resemble sex hormones [34].

3.7. Saponins: Saponins inhibit the growth and viability of cancer cells by interacting with their cholesterol-rich membranes. Pharmacologically, saponins influence key cellular processes related to cell division and growth in humans and exhibit notable anti-inflammatory effects. Therefore, the use of *Amaranthus* spp. leaves is justified in the management of inflammatory conditions [35].

3.8. Tannins: Tannins are recognized for their potential antiviral properties and anticancerous agents [36].

4.Mechanism of endophytes with host plant:

Researchers are fascinated by the bidirectional relationship between plants and endophytes, particularly in understanding how these microbes contribute to the plant's extended phenotype is represented in Fig 3. Endophytes are crucial for plant growth and development, enhancing host defence against pathogens by boosting phytohormone production including salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) [37]. They also strengthen leaf toughness via cellulose deposition, deter herbivorous insects, compete for ecological niches, and secrete hydrolytic enzymes. Additionally, endophytes improve the host plant's resilience to abiotic stresses and increase nutrient accessibility. In exchange, plants offer endophytes a protected habitat along with essential resources such as water and nutrients for their survival and proliferation [38].

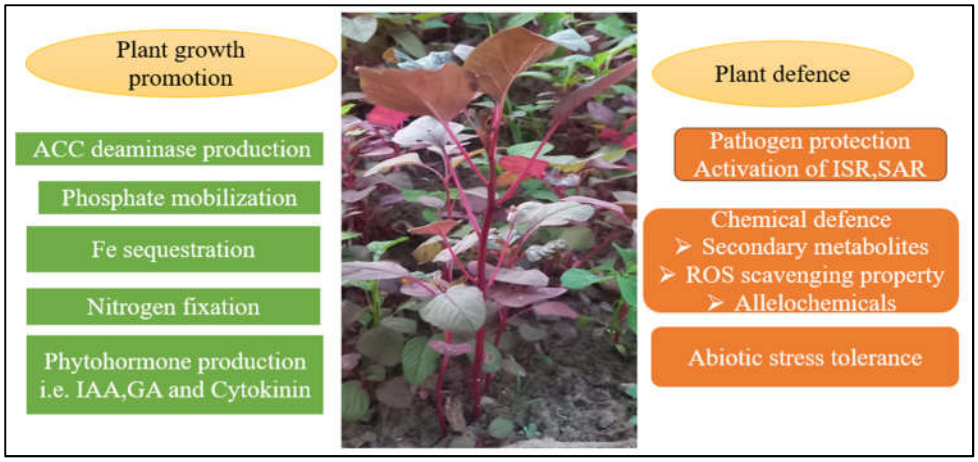

Fig3: Mechanisms of endophytes associated with the host plant (ACC=1-aminocyclopropane-1 carboxylate**,** Fe= Iron, IAA= Indole acetic acid, GA= Gibberellin, ISR= Induced systemic resistance, SAR= Systemic acquired resistance, ROS= Reactive Oxygenic Species)

4.1. Antibiotics production: Producing antibiotics through plant metabolic pathway is considered as an effective method for protecting plants from diseases. Various bioactive compounds can suppress phytopathogens, although only a few have been extensively studied [39]. Endophytes are known to produce a wide range of metabolites, many of which have antimicrobial properties. These include alkaloids, flavonoids, peptides, phenols, polyketides, quinones, steroids, and terpenoids [40,41]. One well-known compound is 2,4-diacetylphloroglucinol (DAPG), a phenolic antibiotic has broad-spectrum antimicrobial activity and plays a significant role in controlling soil-borne plant diseases produced by Pseudomonas species [42].

4.2. 1-Aminocyclopropane-1-carboxylate (ACC)utilization: Ethylene is a crucial phytohormone that regulates normal plant growth and development [43]. It is produced by nearly all plants; ethylene influences various physiological processes and responds to both abiotic and biotic soil factors. However, under stressful conditions, such as pathogen attacks, drought, salinity, or heavy metal toxicity, ethylene levels can rise excessively, leading to detrimental effects like cellular dysfunction, premature leaf drop, and reduced crop yields [44]. To counteract this, certain endophytic bacteria, including species from *Achromobacter, Agrobacterium, Acinetobacter, Bacillus, Enterobacter, Pseudomonas, Serratia, Ralstonia, Rhizobium, Alcaligenes,* and *Burkholderia,* produce the enzyme ACC deaminase [45]. These bacteria degrade ACC (1-aminocyclopropane-1-carboxylate), the immediate precursor of ethylene, into ammonia and 2-oxobutanoate, thereby lowering ethylene levels and alleviating stress [46]. Plants associated with ACC deaminase-producing bacteria can resist diverse stress conditions like radiation, heavy metals, flooding resistance due to stress coming from polyaromatic hydrocarbons, high light intensity, wounds, high salt concentration, insect predation, drought, and extreme temperature. The mechanism of producing IAA and ACC by endophytes diagrammed in Fig 4.

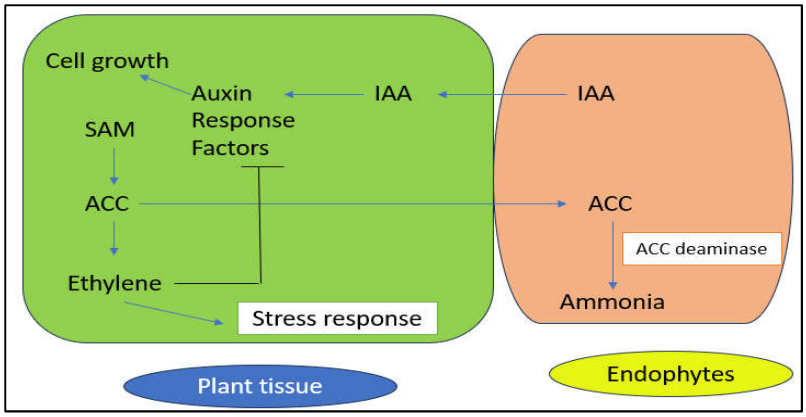

Fig 4: The mechanism of producing both ACC deaminase and IAA by endophytes (SAM= S- Adenosyl Methionine)

4.3. Phytohormone production: Plant hormones play a critical role in regulating various aspects of plant growth, development, and stress responses. Endophytes contribute to plant growth by producing phytohormones within the host plant. For example, some endophytes synthesize indole-3-acetic acid (IAA) [47], which promote plant growth by increasing root surface area and thereby enhancing nutrient uptake from the rhizosphere. Specific endophytes, such as the seed-borne *Bacillus amyloliquefaciens* RWL-1 in rice, and leaf-associated species like *Pseudomonas resinovorans* and *Paenibacill polymyxa*, are known to produce gibberellins (GAs) and cytokinin (CTKs). Additionally, *Piriformospora indica* has been shown to shorten the juvenile phase in black pepper by independently activating GA biosynthesis, photoperiodic, and age-related pathways, ultimately stimulating growth and advancing floral initiation. These findings underscore that successful symbiosis between endophytes and host plants involve precise modulation of hormone biosynthesis pathways and the activation of specific gene clusters responsible for signalling molecule production. Collectively, the studies highlight how endophytes enhance host phytohormone levels, emphasizing the significance of their co-evolution and intricate communication with host plants [41].

4.4. Siderophore production: Siderophore is a low-molecular-weight iron-chelating compound, typically produced by microorganisms like bacteria and fungi, that binds to ferric iron (Fe3+). Plants and microbes need metals like iron, zinc, copper, and nickel to survive, but these nutrients aren't always easy to absorb from the soil. Iron, for example, often gets locked away in forms that plants can't use, leading to poor growth and yellowing leaves. To fix this, plants and their tiny microbial partners called endophytes, release special molecules called siderophores that grab onto iron and make it available for the plant. Different microbes produce different siderophores. Bacteria and fungi make slightly different types, but they all help plants get the iron they need while starving out harmful pathogens [48]. Some siderophores even have antibiotic properties, fighting off dangerous bacteria that attack crops like peanuts and rice. Surprisingly, these iron-grabbing molecules might also help in medicine, like fighting antibiotic-resistant infections, treating malaria, cleaning up toxic metals, and even aiding in cancer therapy.

4.5. Abiotic stress: Plants often face harsh conditions like temperature, high salinity, drought, and nutrient deficiency. Endophytes (microbes living inside plants) can help plants to cope up with these stresses in different ways, such as:

- Producing plant hormones

- Creating helpful compounds
- Activating stress-resistant genes in the plant
- Reducing harmful reactive oxygen molecules

In short, when plants are under stress, endophytes produce useful substances and send signals that turn on stress-response genes or help communicate with other genes that improve tolerance. This helps the plant to survive in harsh conditions [49].

4.6. Biotic stress: Plants face various biotic stresses, like insect attacks which help to boost up the plants defence in different ways. They can release antimicrobial compounds, produce iron-scavenging molecules (siderophores), and activate the plant's immune system i.e., Systemic acquired resistance [49]. The root fungus *Phomopsis liquidambaris* reduces Rice spikelet rot disease by changing the microbial community in rice spikes, increasing helpful bacteria like *Pseudomonas* and Proteobacteria [50]. These bacteria likely enhance defence-related compounds like hordenine and L-aspartic acid. When endophytes colonize plants, they can trigger immune responses similar to those caused by beneficial microbes. For instance, the bacterium *B. cereus* AR156 activates a defence pathway in *Arabidopsis thaliana* that relies on the NPR1 protein and involves jasmonic acid (JA) and ethylene (ET) signalling. These hormones enhance the expression of defence genes (PR1, PR2, PR5) and a key signalling gene (MPK6) [51]. Ethylene (ET) is a stress hormone that helps plants respond to threats, but too much of it harms growth. Endophytes can produce an enzyme (ACC deaminase) that breaks down an ethylene precursor, preventing harmful buildup. Interestingly, if a plant has a mutation that blocks ethylene production, some endophytes can even release ethylene themselves to compensate. This flexibility may come from rapid evolution, allowing endophytes to adapt quickly and help their hosts survive stress. Endophytes fine-tune plant defence in complex ways that avoid triggering strong immune reactions, unlike pathogens [45]. More research is needed to understand how plants recognize endophytes and how these microbes activate immunity against pathogens.

4.7. Enhancing nutrient availability:

Endophytes play a key role in improving plant nutrition. They help plants get more nutrients by either producing them directly or increasing the plant's own nutrient-making processes. This benefits both the endophytes (which get fed) and the plant (which grows bigger and stronger). Some endophytes, like rhizobia in legumes, can convert nitrogen from the air into a form plant can use (ammonium). Even in plants that don't form root nodules (like sugarcane), nitrogen-fixing endophytes do the same job [52]. For example, in finger millet, certain fungi (*Aspergillus terreus*, *Lecanicillium* sp.) and bacteria (*Pseudomonas bijieensis*, *Priestia megaterium*) were found to increase nitrogen, phosphorus, and potassium (NPK) levels in grains. Endophytes also help plants absorb iron by producing siderophores, molecules that grab iron from the soil and deliver it to the plant [53]. Similarly, they break down phosphate in the soil into a form plant can easily take up. Additionally, endophytes enhance photosynthesis by increasing chlorophyll levels and improving energy (NADPH/ATP) production, which helps plants convert carbon dioxide into sugars. Scientists think endophytes help plants get more nutrients to avoid draining the plant's energy. However, more research is needed to understand exactly how endophytes affect plant genes and metabolic pathways. Exploring this could lead to natural ways to improve crop growth and yield [49].

5. Host-Endophyte-Pathogen interaction:

5.1. Host-Endophyte-Pathogen Triangle concept

The "disease triangle" theory, proposed by Russell B. Stevens in the 1960s [54], posits that plant disease development depends on three key factors: the host plant, the pathogen, and the environment. However, recent studies have identified endophytes as a critical fourth component, reshaping this classic model. Unlike the traditional disease triangle, the dynamic interplay between pathogens, endophytes and host plants play a vital role in either suppressing or intensify disease. Endophytes can induce plant resistance against pathogens through direct or indirect mechanisms. Symbiotic microorganisms (including fungi and bacteria) may also coexist with pathogens, sometimes even aiding them in promoting disease [55,56]. In this evolutionary arms race, both plants and pathogens exploit microbial resources to their advantage. The host-pathogen-endophyte interaction is essential for harnessing plant microbiota to develop effective biocontrol strategies against pathogens and is described in Fig 5. Consequently, investigating the genetic regulation within this expanded disease triangle is important.

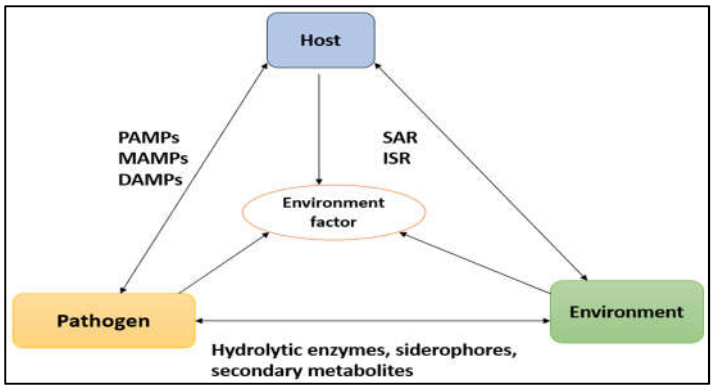

Fig 5: Relationship of Host, pathogen and environment

5.2. Endophytes effectively amplify the plant's immune response:

Plants can actively attract helpful microbes early in their growth to fight off harmful pathogens. The types of microbes that grow in and around the plant are influenced by the plant's own genes. These beneficial microbes, called endophytes, boost up the plant immune system, making it more resistant to diseases [57,58]. When pathogens attack, the microbial communities inside the plant change. This happens because pathogens and endophytes compete for space and nutrients, which can reduce the number of helpful microbes and disrupt their balance [59]. At the same time, interactions between different microbes, whether they can work together or fight each other and also influence whether a plant gets sick [60]. Interestingly, the mix of non-harmful endophytes in a plant is closely linked to how severe disease symptoms become [61]. Some of these microbes, like the *Sphingomonas* bacteria found in *Arabidopsis* leaves, act as a first line of defence against infections [62]. Similarly, when the fungus *Rhizoctonia solani* attacks roots, plants can increase the number of protective bacteria like Chitinaceae and Flavobacteriaceae by turning on specific genes [57]. These bacteria then help prevent root diseases. Endophytes also strengthen plant immunity in other ways. For example, the seed-inhabiting bacterium *B. amyloliquefaciens* RWL-1 helps plants resist disease by adjusting defence-related hormones and amino acids. Beyond that, endophytes can trigger the production of defence enzymes, like phenylalanine ammonia lyase (PAL) [62], which further enhances the plant's resistance.

6. Novel approaches of producing medicines from endophytes

6.1. Endophytic Nanotechnology: Nanoparticles, characterized by their size range of 1–100 nm, possess unique properties that are advantageous for various applications (63). Among the nanoparticle production methods (physical, chemical, and biological), particularly the biological methods involving endophytic fungi, shows significant promise not only for crop improvement and plant health but also an eco-friendly and cost-effective approach which contributes the resilience in synthesis of medicines (64). Nanotechnology is a rapidly advancing field with far-reaching impacts across various aspects of human life (65,66). Among its key components, nanoparticles (NPs) play a pivotal role, particularly in applications like nanomedicine, where they have garnered significant attention (67). Broadly, nanoparticles can be categorized into two groups: organic (e.g., carbon-based nanoparticles) and inorganic (including magnetic nanoparticles, noble metal nanoparticles such as gold and silver, and semiconductor nanoparticles like titanium oxide and zinc oxide).

6.1.1 Drug discovery from nanoparticles: Several studies have reported the antifungal efficacy of biosynthesised nanoparticles (NPs) against *Aspergillus niger*. Biosynthesised NPs exhibited significant antifungal activity against pathogens such as *Phoma glomerata*, *P. herbarum*, *Fusarium semitectum, Trichoderma sp.,* and *Candida albicans*, particularly when combined with the triazole drug fluconazole [68]. In recent years, the rise of antibiotic-resistant microorganisms has growing interest in silver-based antiseptics. Researchers utilised the fungus *Trichoderma viride* to biosynthesize silver nanoparticles (Ag-NPs) [69]. Exposure of aqueous silver (Ag^+) ions to a *T. viride* filtrate led to their reduction, yielding highly stable Ag-NPs ranging from 5 to 40 nm in size. These nanoparticles were then evaluated for their enhanced antimicrobial effects when combined with various antibiotics against both Gram-positive and Gram-negative bacteria [70].

6.1.2 Biosynthesis of nanoparticles by fungi: Fungi are eukaryotic organisms that inhabit diverse environments and often function as decomposers. While an estimated 1.5 million fungal species exist on Earth, only around 70,000 have been identified. However, recent high-throughput sequencing data suggest the actual number may be as high as 5.1 million species [71]. These organisms play a crucial role in breaking down organic matter by secreting enzymes that hydrolyse complex compounds into simpler molecules, which are then absorbed as an energy source [71]. The role of fungi in nanotechnology has gained significant interest, particularly in the biological synthesis of metallic nanoparticles due to their metal tolerance and bioaccumulation abilities [72]. A key advantage of using fungi is their ease of scale-up, such as through solid-substrate fermentation techniques. Additionally, their efficient secretion of extracellular enzymes enables large-scale enzyme production [73]. Fungal-mediated green synthesis of nanoparticles offers economic viability, ease of biomass handling, and simple laboratory cultivation due to the rapid growth of many species [73]. Furthermore, fungi exhibit strong cell wall-binding and intracellular metal uptake capabilities [74]. They can produce metal nanoparticles and nanostructures through extracellular or intracellular enzymatic reduction, as well as biomimetic mineralization processes. Silver nanoparticles (AgNPs) were biosynthesized by incubating an aqueous cell-free filtrate of endophytic fungi with silver nitrate ($AgNO_3$) solution under dark conditions. The reduction of Ag^+ ions to Ag nanoparticles was visually confirmed by a distinct colour change (from yellow to dark orange) in the reaction mixture, accompanied by the emergence of a characteristic surface plasmon resonance (SPR) absorption band between 400–450 nm in UV-VIS spectroscopy. No such colour shift or spectral peak was observed in the control (cell-free supernatant alone). All biological metallic nanoparticle (AuNPs, PtNPs, TiNPs etc.) synthesis procedure is same [75,76]. The synthesized

endophytic fungi NPs were isolated via centrifugation, followed by washing with deionized water to remove unreacted precursors. Subsequent UV-VIS analysis of the purified nanoparticles revealed a stable SPR peak at 439 nm, consistent with the typical plasmonic signature of spherical silver nanoparticles [76]. Different advantages of nanoparticles by fungi and the nanoparticle synthesis procedure are represented in Fig 6 and Fig 7 respectively.

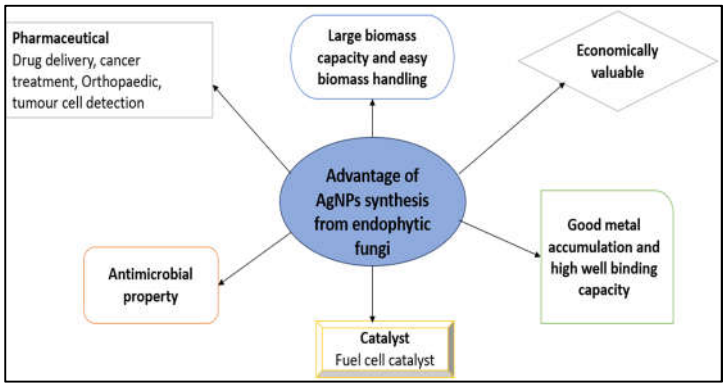

Fig 6: Utilization of Nanoparticle synthesis by fungi

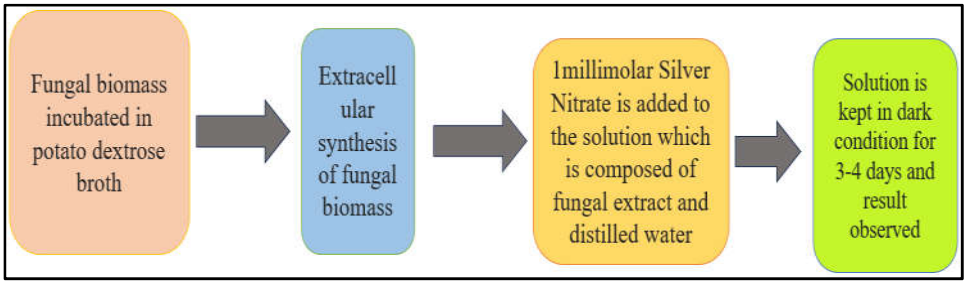

Fig 7.: Synthesis of NPs from endophytic fungi

6.2. LC-MS: The discovery of antifungal drugs is a dynamic field that continually yields promising new candidates, several of which advances to clinical trials. According to Prescott et al. 2023 [77], recently developed compounds that have progressed to first level trial. While not all of these candidates will ultimately gain approval, their advancement to clinical testing reflects strong in vitro performance and therapeutic potential. The need of development of new clinically effective drugs from fungi has explored large-scale drug discovery based mainly on extensive fungal strain. In this traditional approach, environmental samples including soil, water, and plant matter, are collected, then isolation and genotyping of individual fungal strains are done. These isolates are then cultured on various media to promote secondary metabolite production. Subsequent extraction of fungal mycelium or culture broths allows for bioactivity screening, with LC-MS used to exclude known compounds. For example, Novartis, one of the few major pharmaceutical companies still actively engaged in fungal natural product research which maintains a vast and expanding collection of tens of thousands of fungal strains, sourced globally. Strains showing promising bioactivity in initial screenings are re-cultured at an industrial scale, often requiring tens of thousands of litres of growth media and organic solvents for extraction. Despite these efforts, chemical purification typically yields small amount of pure compound. While this approach has led to innovative drugs but it has some disadvantages. Some fungi grow too slowly or resist cultivation altogether. Moreover, many harbour silent

biosynthetic gene clusters that remain inactive under standard laboratory conditions, evidenced by the gap between predicted and observed secondary metabolites [78]. These clusters may only activate during interactions with host plants or competing fungi, suggesting that conventional culture methods overlook a wealth of potential bioactive compounds.

6.3 Hexagon Bio: Recently, new startup like Hexagon Bio have emerged to optimize fungal drug discovery through innovative strategies. Their approach offers two key advancements. First, they employ a tailored library of non-overlapping promoter sequences in *Saccharomyces cerevisiae* to heterologously express silent biosynthetic genes, bypassing the need for laborious culture optimization to induce metabolite production [79]. Second, they exploit fungal self-resistance mechanisms to rapidly pinpoint biosynthetic gene clusters and even predict metabolite function before chemical isolation [80]. For instance, if screening identified *Aspergillus terreus* (the producer of lovastatin) without prior knowledge of its metabolites, the presence of a duplicated HMG-CoA reductase gene in its biosynthetic cluster would immediately suggest the fungus synthesizes a compound targeting fatty acid metabolism, mirroring lovastatin's mechanism.

This strategy significantly accelerates drug discovery by enabling researchers to predict a metabolite's mechanism of action before identifying the compound itself. For example, since HMG-CoA reductase is a clinically validated drug target with a human counterpart, Hexagon Bio can directly clone the corresponding fungal biosynthetic genes into yeast using their engineered promoters and rapidly characterize the metabolite. In contrast, traditional methods would require large-scale cultivation of *A. terreus* in optimized media to produce lovastatin, followed by laborious chemical isolation and mode-of-action studies. A yeast chemical genetic assay where hypersensitivity of an HMG-CoA reductase deletion strain to the compound would reveal its target it could then confirm the mechanism. The duplicated HMG-CoA reductase gene in the fungal cluster elegantly bypasses this time-consuming process, offering a shortcut past a major bottleneck in conventional discovery.

Focusing on self-resistance genes offers another key advantage: it inherently selects for compounds with specific, targeted mechanisms of action. Only molecules that selectively bind a particular receptor can employ gene duplication for self-resistance, whereas non-selective "junk compounds" (e.g., those causing general DNA intercalation or membrane disruption) are filtered out. This provides a major efficiency boost up by eliminating the majority of false-positive "bioactive" natural products reported in screening studies. However, this approach has one critical limitation, it would miss compounds targeting human receptors without fungal homologs. These would still require traditional isolation and testing. Ultimately, combining both strategies may be essential to maximize the discovery of potential blockbuster drugs [77].

6.4 Traditional and scientific methods: Recent years have witnessed growing interest in plant-endophytic associations as sources of high-value metabolites with pharmacological potential. Deep learning approaches have emerged as powerful tools for endophyte bioprospecting, enabling the prediction of novel chemical entities and analysis of regulatory networks [81]. While endophytes represent attractive biosynthetic platforms for novel bioactive compounds, significant challenges remain in their exploitation for drug discovery. A major limitation lies in the frequent silencing of secondary metabolite pathways, coupled with our incomplete understanding of the underlying metabolic networks and regulatory mechanisms. Computational predictions further suggest that a wealth of potentially valuable metabolites remain silent under normal growth conditions [82,83].

Traditional approaches to natural product discovery from endophytes have relied on conventional low-throughput methods including culture-based isolation, plant extraction techniques, and bioactivity-guided fractionation using HPLC, NMR and MS [84,85]. To overcome these limitations, researchers have developed more cultured strategies. The OSMAC (one strain-many compounds) approach has proven particularly valuable, where varying culture conditions can stimulate production of diverse metabolites from a single strain [83,86]. Other favourable techniques include ribosome engineering, heterologous gene expression, and promoter manipulation, all aimed at activating silent biosynthetic pathways through biotic or abiotic stimulation.

6.5 Genetic approaches: These have similarly enabled activation of silent biosynthetic gene clusters (BGCs) through methods such as inducible promoter systems, ribosome engineering, and mutant selection [87,88]. For culturable endophytic bacteria, high-throughput techniques like imaging mass spectrometry-based elicitor screening have shown potential for inducing silent gene clusters [89], these methods have limitations to cultivable microorganisms. This limitation underscores the need for continued development of cultivation-independent approaches to fully access the metabolic potential of plant-associated endophytes.

6.6 Endophytic fungal drug discovery for chronic disease: Endophytic Fungi have yielded some of the most groundbreaking drugs in medical history, including both historically significant treatments and modern blockbuster medications. These fungal-derived compounds play critical roles in managing chronic conditions, particularly chronic infections (antibiotics and antifungals), autoimmune disorders (immunosuppressants), and hypercholesterolemia (statins). More recently, metabolites sourced from fungi have advanced into clinical trials for chronic diseases such as cancer and treatment-resistant depression. In this review, we explore the discovery timeline of well-established fungal-based drugs and evaluate promising new compounds undergoing clinical testing. While definitions of chronic disease vary, we adopt the CDC's criteria, which characterize chronic diseases as persistent conditions lasting at least one year, requiring continuous medical care, restricting daily activities, or both [90].

The recognition that fungi produce biologically active compounds is far from novel, predating modern scientific methods. Various cultures have historically utilized fungi for medicinal and hallucinogenic purposes [91]. Similarly, the isolation of pure chemical compounds from fungi is not a recent development, with documented examples tracing back to at least 1893[92]. Despite the quick awareness of fungal bioactivity, the present era of fungal drug discovery began with penicillin. This breakthrough captured the scientific community's attention, revealing fungi as a rich reservoir of therapeutic small molecules. A serendipitous observation, a mold contaminant suppressing bacterial growth on a Petri dish, led to the isolation of an extraordinarily effective antibiotic that would reshape medical history.

The next landmark antibiotic, cephalosporin C, shared penicillin's β-lactam core and mechanism of action. Its producer, *Acremonium chrysogenum*, was isolated in 1945 by Giuseppe Brotzu from Sardinian seawater near a sewage outlet, where he investigated the puzzling rarity of Salmonella infections among swimmers [93]. Brotzu hypothesized microbial "self-purification" and identified an antifungal strain exhibiting antibiotic properties. This spurred the development of cephalosporins, which remain crucial against penicillin-resistant bacteria. Both penicillin and cephalosporin discoveries were hampered by limited separation technologies, prompting reflection: could earlier clinical use have been achieved with semi-purified fractions as topical agents it inhibits bacterial protein synthesis without affecting eukaryotic translation and making it utilizable for topical skin and eye infections [94].

Less commonly cited is fusafungine, an antimicrobial and anti-inflammatory enniatin mixture from *Fusarium lateritium*, used for respiratory infections until its 2016 withdrawal due to allergy risks (95,96). Meanwhile, the antibiotic resistance crisis has revived interest in overlooked fungal compounds like pleuromutilin, first isolated from *Clitopilus scyphoides* in the 1950s. Their synthetic derivative, retapamulin, was approved in 2007 for skin infections [97]. Fungi also produce antifungal drugs. Griseofulvin, isolated from *Penicillium griseofulvum* in 1938, used in treatment of skin diseases by disrupting microtubule function. More recently, echincandins, semisynthetic inhibitors of fungal cell wall synthesis (e.g., caspofungin, micafungin, anidulafungin) have become first-line therapies for invasive mycoses [98]. Caspofungin, derived from *Glarealozoyensis* (discovered in a Spanish farm pond (99), was approved in 2001, followed by micafungin (from *Coleophomaempedra*) and anidulafungin (from *Aspergillus spinulosprous*) [98,99]. A 2000 discovery, enfumafungin, a triterpene glycoside from the endophyte *Endoconidio macarpetanum* yielded ibrexafungerp, approved in 2021 for vulvovaginal *C. andidiasis*. Despite these advances, the rise of pathogens like Candida auris underscores the need for antifungals with novel mechanisms to combat resistance [100].

Fungi have also proven to be a remarkably productive source of immunosuppressant compounds. The first such drug, mycophenolic acid, was isolated in 1893 from *Penicillium brevicompactum* found in spoiled corn silage, though its clinical application came much later [92]. This compound works by inhibiting inosine monophosphate dehydrogenase (IMPDH), thereby blocking guanine biosynthesis and subsequent lymphocyte proliferation. It has become a mainstay in organ transplantation, with its synthetic derivative, mycophenolate mofetil, approved in 1995 for kidney transplants. A delayed-release formulation developed by Novartis achieved $441 million in annual sales by 2015 [101]. Similarly, mizoribine, isolated in 1971 from *Penicillium dodgei* (formerly *P. brefeldianum*) in a soil sample from Japan's Hachijo Island, also targets IMPDH and has been used in Japan since 1984 to prevent renal transplant rejection [102].

Remarkably, two groundbreaking immunosuppressants originate from entomopathogenic fungi. Cyclosporin A, derived from *Tolypocladium inflatum* (a fungus that parasitizes beetle larvae), revolutionized organ transplantation by inhibiting T-cell activation via the calcineurin pathway [103]. Before its introduction, organ transplants were largely experimental, with most patients surviving only weeks post-surgery. Like penicillin, cyclosporine redefined medical possibilities. Fungi have also transformed cholesterol management. In the 1970s, Akira Endo's screening of 6,000 fungal extracts led to the discovery of mevastatin (compactin) from *Penicillium citrinum*, a mold infecting Japanese oranges (Lovastatin, later isolated from Monascusruber (as monacolin K) and *Aspergillus terreus* (as mevinolin), became the first FDA-approved statin in 1987[104]. By competitively inhibiting HMG-CoA reductase [104]. The ergot alkaloids, produced by *Claviceps purpurea* on cereal crops, have a darker history. Ergotism, caused by consuming infected rye, led to epidemics like St. Anthony's Fire in medieval Europe, marked by hallucinations and convulsions [105]. Yet, these toxins also yielded medicines: ergotamine (isolated in 1918) treats migraines, while ergometrine maleate manages postpartum haemorrhage by inducing uterine contractions.

7. Conclusions: Endophytes play a crucial role in enhancing plant health and resilience, particularly in the *Amaranthus* genus, which offers significant nutritional, medicinal and economic benefits. Amaranth possesses advantageous physical and chemical properties. Despite its superior nutritional profile and competitive edge over conventional grains, amaranth remains underutilized. This study demonstrates its significant value as a rich source of energy, starch, protein, fibre, vitamin E isomers, and beneficial secondary metabolites. The disease

triangle methods and plant's immune response with mutualistic association with endophytes are discussed here. Endophytes also act as potential source of novel medicines and different traditional and genetic approaches are described here for healing different chronic diseases. A number of scientific approaches like LC, Nanotechnology, OSMAC approach also discussed here for production of new drugs. Biological synthesis of nanoparticles by utilizing endophytes decreases environmental threats as well as increases better human immune response. But, some technologies from endophytes producing drugs are under the process. This endophyte mediated medicine productions opens a new door in pharmaceutical industry and also possesses a non-hazardous way and environmentally friendly which have lower side effects. By recognizing the potential of *Amaranthus* as a climate-smart crop, we can address food insecurity while promoting sustainable agricultural practices and exploring its diverse applications in health, nutrition, new medicine and other ecological uses.

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