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An Overview of the Uses and Pathogenesis of Endophytic Fungi Isolated from Plants and Vegetables

Omali Ebele Mediatrix and Nwiboko Wilfred

Department of Hospitality NIHOTOUR Enugu, Nigeria

ABSTRACT

Endophytes are micro-organisms that inhabit plant hosts for all or part of their life cycle. They inhabit the inner plant tissues underneath the epidermal cell layers without causing any obvious damage or indicative infection to their host, living within the intercellular spaces of the tissues.Fungi constitute the largest number of plant pathogens and are responsible for a range of serious plant diseases and infections. Most vegetable diseases are caused by fungi. They damage plants by killing cells and/or causing plant stress. Some sources of fungal infections are infected seed, soil, crop debris, nearby crops and weeds. Fungi are spread by wind and water splash, and through the movement of contaminated soil, animals, workers, machinery, tools, seedlings and other plant material. They enter plants through natural openings such as stomata and through wounds caused by pruning, harvesting, hail, insects, other diseases, and mechanical damage.Some of the fungi are responsible for foliar diseases – Downy mildews; Powdery mildews; and White blister are some of the highly prevalent foliar diseases. Secondly, some endophytic fungi would produce different bioactive compounds, such as alkaloids, diterpenes, flavonoids, and isoflavonoids, to increase the resistance to biotic and abiotic stresses of their host plants. Thirdly, some endophytic fungi could promote the accumulation of secondary metabolites (including important medicinal components or drugs) originally produced by plants.Some endophytic fungi could increase the fitness and growth of host plants by increasing hormones, such as indole-3-acetic acid, indole-3-acetonitrile, and cytokinins. Endophytic fungi could also promote the growth of their host plants by obtaining nutritional elements such as nitrogen and phosphorus useful for plants. So in conclusion, endophytic fungi isolated from plants and vegetables have been shown to confer some beneficiary and pathogenic effects as enumarated on this research.
Keywords: Endophytes, micro-organisms, plants, vegetables, fungi and diseases.

INTRODUCTION

The word endophytes encompasses a family of micro-organisms that grows intracellular (within the cell) or intercellular outside the cell in the tissues of higher plants without any symptoms on the plants in which they reside [1]. Endophytes are the micro-organisms residing in the internal tissues of the plants in a symbiotic relationship without any apparent symptoms of infections [2]. biological research on endophyte is an on going research in mycology field.[3], this special ecological niche together with the constant metabolic exchanges between the fungus and the plant seems to serve as a strong evolutionary pressure for the endophytes to produce secondary metabolites [4], which could advance the fitness of the host plant and its resistance to various pests [5]. In addition, it has also been revealed that the produced metabolites are intermittently the same as those, which have initially been isolated and identified from the plant hosts [6]. This examination promoted the isolation of several fungal endophytes producing important medicinal agents including digoxin, ginkgolides and podophyllotoxin originally described from *Digitalis lanata* [7], *Ginkgo biloba* [8] and *Juniperuscommunis* [9], respectively, as well as vincamine and vinpocetine isolated initially from *Vinca minor* [10].

They inhabit the inner plant tissues underneath the epidermal cell layers without causing any obviousdamage or indicative infection to their host, living within the intercellular spaces of the tissues [11]. It seems that they may infiltrate the living cells when taken. Endophytes form unremarkable infections within tissues of healthy plants for all or nearly all their life cycle [12]. Also, their host tissues appear symptomless, and they remain asymptomatic for many years and only become parasitic when their hosts are stressed [13]. The term "Endophyte" initially was coined by German scientist Heinrich Anton De Bray in 1884 [14]. Endophytic fungus was originally identified by Freeman in 1904, and was isolated from *Loliumpersicum* [15].

Approximately, there are near to 320,000 plant species on earth and every individual plant is the host to one or more endophytes, and many of them may colonize certain hosts [16]. It has been estimated that there may be as many as one million plusdifferent endophytic fungal taxa, thus endophytes may be hyperdiverse [17]. Endophytic fungal communities are influenced by many factors such as geographical location, climatic patterns, physiology and specificity of colonized tissue [18]. They are present in most plant parts, especially the leaves, where the tissue is apparently healthy. Endophytes relationship with plant varies from symbiotic to pathogenic. Endophytes benefit host plant by preventing pathogenic organism from colonizing [19]. It has been categorized into two main groups (Clavicipitaceous and non-clavicipitaceous) based on differences in evolution, taxonomy, plants host and ecological functions. Clavicipitaceous are able to infect only some species of grasses and nonclavicipitaceous are found in the asymptomatic tissues of other higher plants [20]. It stimulates plants growth, increase disease resistance, improve the plants ability to withstand environmental stresses and recycle nutrients.

Relationship between Endophytic Fungi and Medicinal Plants

It is broadly considered in a conventional research that the quality and quantity of crude drugs originated from medicinal plants are largely affected by such factors as the genetic environment of the concerned plants, ecological habitats where the plants live, and soil nutrients [21]. Nevertheless, in the current years, it is progressively known that endophytic fungi or endophytes have played a very important role in affecting the quality and quantity of the crude drugs through a particular fungus-host interaction, indicating that more understanding on the particular relationships between endophytic fungi and medicinal plants is required for promoting crude drug production [22].Though, endophytic fungi are one of the most important elements in plant micro-ecosystems that should have significant influences on the growth and development of host plants, our knowledge about the exact relationships between endophytic fungi and their host plants is still very limited [23]. Appreciating and exploiting such relationships will smooth the progress of the ideal production of improved drugs by manipulating the growth conditions of medicinal plants by, for example, adding a particular group of endophytic fungi to the plants to improve the drug quality and quantity [24].

Schultz classified the fungal endophytic fungi into three main ecological groups: (a) mycorrizal; (b) balansicaeous or pasture endophytic fungi; and (c) non pasture endophytic fungi [25]. The bioactive compounds produced by endophytic fungi, exclusive of those to their host plants, are very important to increase the adaptability of both endophytic fungi and their host plants, such as the tolerances to biotic and abiotic stresses. In addition, these compounds can induce the production of a plethora of known and novel biologically active secondary metabolites that can be exploited and applied by human as important medicinal resources [26]. It is known that the colonization of endophytic fungi is not an opportunity because of the chemotaxis that is specific chemicals produced by the host plants [27]. At the same time, different types of secondary metabolites, such as saponin and essential oils from medicinal plants, are produced through long-term co-evolution as a resistance mechanism to the pathogens, most possibly including endophytic fungi. Therefore, the secondary metabolites became obstacles for the colonization of endophytic fungi. To overcome this, endophytic fungi must secrete the matching detoxification enzymes, such as cellulases, lactase, xylanase, and protease, to decompose these secondary metabolites before they penetrate through the defense systems of the resided host-plants. Once inside the tissues of a host-plant, the endophytic fungi assumed a quiescent (latent) state, either for the whole lifetime of the host plant (neutralism) or for an extended period of time (mutualism or antagonism) until environmental conditions are favorable for endophytic fungi or the ontogenetic state of the host changes to the advantage of the fungi [28].

At some stage in the long period of co-existence and evolutionary processes, diverse relationships have been recognized between endophytic fungi and their host plants through a particular fungus-host interaction recognized as: (i) a continuum of mutualism, (ii) antagonism, and (iii) neutralism [29]. The genetic background, nutrient level, and ecological habitats of the medicinal host plants are considered as the pressure-choice factors on the population structure of the endophytic fungi that, in turn, confer some kinds of benefits, such as the induced growth, increased resistance to disease, and/or herbivore as well as accumulated bioactive components [30], some of which can be used by human as beneficial medicines. Therefore, the mutual interrelation between endophytic fungi and their host plants can impose certain effects on the formulation of some types of bioactive compounds that can be used by human.

Fungal diseases in vegetable crops

Fungi constitute the largest number of plant pathogens and are responsible for a range of serious plant diseases [10]. Most vegetable diseases are caused by fungi [11]. They damage plants by killing cells and/or causing plant stress. Sources of fungal infections are infected seed, soil, crop debris, nearby crops and weeds as earlier stated, Fungi are spread by wind and water splash, and through the movement of contaminated soil, animals, workers, machinery, tools, seedlings and other plant material. They enter plants through natural openings such as stomata and through wounds caused by pruning, harvesting, hail, insects, other diseases, and mechanical damage. Some of the fungi are responsible for foliar diseases – Downy mildews; Powdery mildews; and White blister are some of the highly prevalent foliar diseases. Other fungi – Clubroot; Pythium species; Fusarium species; Rhizoctonia species; Sclerotinia and Sclerotium species – are soilborne diseases. Some fungal diseases occur on a wide range of vegetables. These diseases include Anthracnose; Botrytis rots; Downy mildews; Fusarium rots; Powdery mildews; Rusts; Rhizoctonia rots; Sclerotinia rots; Sclerotium rots. Others are specific to a particular crop group, e.g. Clubroot (*Plasmodiophorabrassicae*) in brassicas, Leaf blight (*Alternariadauci*) in carrots, and Red root complex in beans [15].

Common fungal diseases and crops affected

Table 1: Some examples of common fungal diseases of vegetable crops are provided in the table below with some typical symptoms.

Fungal disease	Factors conducive to spread	Crops affected	Symptoms
White blister/White rust (*Albugo candida*)	Optimum conditions for disease development are 3-4 hours in mild temperatures (6- 24 °C)	Brassicas (including Asian leafy brassicas).	White blisters and swellings on leaves and heads of affected plants; blisters consist of masses of white dust-like spores; up to 100% losses have been reported.
Downy mildews (individual species damage particular crop families)	High humidity, leaf wetness and cool to mild temperatures (10-16 °C).	Wide host range including onions; peas; lettuce; celery; spinach; kale; herbs; cucurbits; brassicas; Asian leafy brassicas.	Symptoms usually begin with yellowish leaf spots which then turn brown; downy growth appears on underside of leaves.
Powdery mildews (some species are restricted to particular crops or crop families)	Moderate temperatures (20-25?C); relatively dry conditions (unlike downy mildews).	Wide host range and very common, especially in greenhouse crops: cucumber; melons; pumpkin; zucchini;parsnip; beetroot; potato; herbs; peas; bitter melon;tomato; capsicum; Brussels sprouts; cabbage; swedes.	Small, white, powdery patches on most above-ground surfaces; usually observed first on undersides of leaves but eventually cover both surfaces; affected leaves become yellow, then brown and papery and die.
Clubroot (*Plasmodiophorabrassicae*)	Warm weather; acidic soil	Brassicas (including Asian leafy brassicas).	Plants are yellow and stunted and

	(pH less than 7); high soil moisture.		may wilt in hotter part of the day; large malformed 'clubbed' roots which prevent the uptake of water and nutrients, reducing the potential yield of the crop.
***Pythium* species**	Cold, wet soil conditions; known as water moulds, they enter untreated water supplies; water supplies for irrigation and hydroponics should be tested regularly.	Many vegetable crops in including cucurbits; brassicas; lettuce.	May kill seedlings, which die before they emerge or soon after emergence; plant collapse.
Sclerotinia rots (*S. sclerotiorum* and *S. minor*) – a range of common names are used	Windy, cool, humid weather; wet soil; survival structures known as sclerotia remain viable in soil for long periods (10-15 years).	Most vegetable crops.	Water-soaked rotting of stems, leaves, and sometimes fruit; followed by a fluffy, white and cottony fungal growth which contain hard black pebble-like sclerotia.
Sclerotium rots (*Sclerotiumrolfsii* and *S. cepivorum*)	*S. rolfsii* – Warm, moist conditions. *S. cepivorum* – Development is favoured by cool soil conditions (14-19?C) and low moisture.	*S. rolfsii* – Wide host range including: beans; beets; carrot; potato; tomato; capsicum; cucurbits. *S. cepivorum* – only affects onions, garlic and related Alliums (shallots; spring onions; leeks).	*S. rolfsii* – Lower stem and root rots; coarse threads of white fungal growth surround the diseased areas; small brown fungal resting bodies. *S. cepivorum* –

			Yellowing and wilting; fluffy fungal growth containing black sclerotia forms at the bases of bulbs.
Fusarium wilts and rots (Various Fusarium species including *F. solani* and *F. oxysporum*)	Warm to hot weather.	Wide host range including: brassicas; carrots; cucurbits;onions; spring onions; potato; tomato; herbs; peas; beans.	Causes severe root and crown rots or wilt diseases by attacking roots and basal stems; cucurbit fruit and potato tubers can be affected in storage.
Botrytis rots – for example Grey mould (*Botrytis cinerea*)	Cool, wet weather.	Celery; lettuce; beans; brassicas; cucumber; capsicum; tomato.	Softening of plant tissues in the presence of grey fungal growth.
Anthracnose (*Colletotrichum spp.* except for in lettuce – *Microdochiumpanattonianum*)	Cool, wet conditions.	Wide range of crops including: lettuce; celery; beans; cucurbits; tomato, capsicum; potato; globe artichoke.	Typical symptoms begin with sunken and water-soaked spots appearing on leaves, stems and/or fruit.
Rhizoctonia rots (*Rhizoctoniasolani*) – range of common names, e.g. Bottom rot (lettuce) and Wire stem (Brassicas)	Warm, humid weather; can survive for long periods in the soil in the absence of a host plant.	Wide host range including: lettuce; potato; brassicas;beans; peas; beets; carrots; capsicum; tomato; cucurbits.	Range of symptoms depending on the crop being grown but can affect roots, leaves, stems, tubers and fruit; plants wilt and may collapse and die.
Damping-off (Pythium, Rhizoctonia, Phytophthora, Fusarium or Aphanomyces)	Occurs under cold, wet soil conditions; shore flies and fungus gnats can spread Pythium and Fusarium.	Many vegetable crops including: leafy vegetables;brassicas ; carrots; beetroot; cucurbits, eggplant; tomato;coriander; spring onions; beans	Young seedlings have necrotic stems or roots; seedlings die or show a reduction in growth.

Cavity spot (Pythiumsulcatum)	Growing carrots after carrots; acidic soil; not harvesting carrots as soon as they reach marketable size.	Carrots.	Cavity spots are small elliptical lesions often surrounded by a yellow halo.
Tuber diseases (Various species)		Potato and sweetpotato.	Potato tubers may be infected with superficial skin diseases, such as common scabs, powdery scab, and Rhizoctonia. Sweetpotatoes may be infected by scurf.
Rusts (several species, e.g. *Pucciniasorghi* – sweet corn; *Uromycesappendiculatus* – beans; *Pucciniaallii* – spring onions).	Wind can spread spores great distances; favoured by low rainfall, 100% relative humidity and cool to mild temperatures.	Sweet corn; beans; onions; spring onions; beets; celery; silverbeet; endive.	Small, red or reddish-brown pustules that form on the underside of the leaves and sometimes on the pods as well; dusty reddish-brown spores released from pustules (may be black in cold weather).
Black root rot (Different species on different vegetable crops)	Cool soil temperatures; high soil moisture.	Lettuce; beans; cucurbits.	Blackening of roots; stunted plants; plants may die.

Other fungal diseases of vegetables include:

Target spot – *Alternariasolani* (tomatoes).
Aphanomyces root rot – *Aphanomyceseuteichespv. phaseoli* (beans).
Aschochyta collar rot (peas).
Gummy stem blight – *Didymellabryoniae* (cucurbits).
Alternaria leaf spot – *Alternariacucumerina* and *A. alternata* (cucurbits).
Black leg – *Leptosphaeriamaculans* (brassicas).
Ring spot – *Mycosphaerellabrassicicola* (brassicas).
Late blight – *Septoriaapiicola* (celery).
Cercospora leaf spot – *Cercosporabeticola* (beets).
Leaf blight – *Septoriapetroelini* (parsley).
Septoria spot – *Septorialactucae* (lettuce).
Leaf blight – *Stemphyliumvesicarium* (spring onions).
Leaf blight – *Alternariadauci* (carrots).

Management of fungal diseases in crops

The following procedures are used in the management of fungi diseases:

i. Understand the lifecycles, survival mechanisms, and conducive environmental conditions for fungi.
ii. Be committed to farm sanitation – clean up your farm and remove all weeds, crop debris, and volunteer hosts.
iii. Use resistant or tolerant varieties.
iv. Use clean transplants and seed (and seed treatments).
v. Monitor weather conditions (particularly temperature, humidity, and leaf wetness).
vi. Have knowledge of relevant disease prediction models.
vii. Understand the implications for irrigation timing and minimise free moisture and high humidity periods (e.g. irrigating at around 4 am rather than at dusk, not irrigating during peak periods of spore release).
viii. Appropriate crop rotations (long rotations with non-host crops may be necessary).
ix. Avoid heavily infested blocks by testing soil for soilborne diseases prior to planting.
x. Monitor crops regularly and be able to detect early symptoms on your crop.
xi. Amend and manage soil to disadvantage the fungi (some fungal diseases can survive in the soil for 30 years or more).
xii. Minimise ways in which the disease can spread on-farm – remove and destroy sick plants when symptoms first show.
xiii. Understand the influence of planting time, plant spacing and overlapping crops.
xiv. Apply preventative fungicides based on weather conditions.
xv. Understand fungicide resistance and rotation of chemical groups.

Beneficial Relationships Conferred by Endophytic Fungi to Host Plants

Analysis based on the certain references additionally indicated the benefits conferred by some endophytic fungi to their host plants after colonization. Firstly, some endophytic fungi could produce different plant hormones to enhance the growth of their host plants [21]. For example, the growth of wheat (*Triticumaestivum* L.) could be enhanced by *Azospirillum* sp. under drought stresses [22]. Secondly, some endophytic fungi would produce different bioactive compounds, such as alkaloids, diterpenes, flavonoids, and isoflavonoids, to increase the resistance to biotic and abiotic stresses of their host plants [22]. Thirdly, some endophytic fungi could promote the accumulation of secondary metabolites (including important medicinal components or drugs) originally produced by plants. These metabolites may be produced by both of the host plants or/and endophytic fungi according to the references surveyed [23], 24], [25], [26], [27]. Owning to the importance of the three aspects, we would present the three types of possible beneficial endophytic fungus-host relationships accordingly.

Promotion of Fitness and Growth of Host Plants

Some endophytic fungi could increase the fitness and growth of host plants by increasing hormones, such as indole-3-acetic acid, indole-3-acetonitrile, and cytokinins. Endophytic fungi could also promote the growth of their host plants by obtaining nutritional elements such as nitrogen and phosphorus useful for plants.

For example, *Mycenadendrobii* could promote the seed germination and growth of the host plant *Gastrodiaelata* by secreting indoleacetic acid. In addition, *Metarhiziumrobertsii* transloca ted nitrogen directly from insects to its host plants through hyphae [28]. Interestingly, results showed that most hormones were produced by endophytic fungi isolated from the roots of host plants. A few references also reported that some endophytic fungi could promote the growth and fitness of the host plants by activating the expression of a certain enzymes and genes. For example, *Piriformosporaindica* increased the growth of tobacco roots by stimulating the expression of nitrate reductase and the starch-degrading enzyme (glucan-water dikinase).

Increase of Resistance to Stresses for Host Plants

The references showed that certain endophytic fungi could enhance the resistance of host plants to biotic and abiotic stresses by producing bioactive compounds (chemicals) [20]. In symbiotically conferred stress tolerance, the endophytic fungi were considered to act as a type of biological trigger that activated the defense systems of a host. For example, endophytic fungi that were inoculated to crop plants improved the resistance and yield of the crops and such anendophytic-mediated plant resistance to pathogens was more likely the result of direct competition between host plants and pathogens.Moreover, chemicals produced by endophytic fungi were toxic or distasteful to insects protecting the host plants from the attacks of insects. For example, alkaloids produced by endophytic fungi in the genus *Neotyphodium* could confer deterrence to their host plants, increasing their survival from the attacks by insects. With the increased stress tolerance, host plants infected by endophytic fungi could outcompete native plants without fungal infection, and consequently became invasive.

In addition, endophytic fungi could produce a vast variety of antioxidant compounds that could protect their hosts by enhancing tolerance to abiotic stresses. In supporting of this, several studies had demonstrated increased production of antioxidant compounds (e.g., flavonoids and other phenolic antioxidants) in endophyte-infected plants. Furthermore, it was shown that endophytic fungi possessing metal sequestration or chelation systems were able to increase tolerances of their host plants to the presence of heavy metals, thereby, assisting their hosts to survive in contaminated soil [8].

Promoting the Accumulation of Bioactive Compounds of Medicinal Plants

Results from our reference analyses clearly indicated that some endophytic fungi has the ability to promote the accumulation of secondary metabolites of host plants, which influenced the quantity and quality of drugs [16]. Some endophytic fungi could produce diverse classes of phytochemicals—secondary metabolites originally from plants, including the well-known compounds such as paclitaxel (also known as taxol), podophyllotoxin, deoxypodophyllotoxin, camptothecin, and structural analogs, hypericin and emodin and azadirachtin. In fact, the best known example of anticancer compound taxol was found in the taxol-producing endophytic fungi *T. andreanae* that was isolated from *T. Brevifolia*. Many endophytic fungi colonized in other host plant species, such as *Seimatoantleriumtepuiense, Seimatoantleriumnepalense* (*Tuberculari a* sp. strain were also found to produce taxol [20].Other endophytic fungi could promote the formation and accumulation of secondary metabolites that were only produced by host plants. Forexample, *Coetotrichumgloesporioides* could induce the production of Artemisinin in hairy-root cultures of *Artemisia annua* .These compounds commonly function as bioactivities for antitumor, antipyretic, antimalarial, analgesic, or anti-inflammatory in medicinal treatments.

DISCUSSION

The plant tissues, especially leaves and stems are excellent reservoirs for endophytic fungi. Environmental factors such as temperature, rainfall and atmospheric humidity and their effect on host plant made the variations in occurrence of endophytic fungi and their colonization frequency [2].The present study was based on the observation that certain endophytes are able to produce the same metabolites as their plant hosts and thus, they can serve as novel microbial sources of bioactive plant metabolites [6]. Hypericin is one of the medicinally important polyphenolic compounds as it has been proven to have antidepressant, antitumor and antiviral properties and it is also used in photodynamic therapy for the detection and treatment of tumor cells [7].

This review highlights the environmental and host-plant factors that can possibly influence the population structure and distribution of endophytic fungi, as well as the benefits these endophtes provide to their host plants [14].

The fungus-host relationships reveal that the distribution and population structure of endophytic fungi rely largely on the taxonomy, genetic background, age, and tissues of the host plants, in addition to the types of environments [20]. These findings can assist in the investigation of bioactive compounds produced by a certain host medicinal plant under specific environment conditions. In addition, we have observed that there are three types of beneficial interactions between endophytic fungi and their host plants namely: (1) enhancement of the growth of host medicinal plants, (2) increase in the resistance of the host plants to biotic and abiotic stresses, and (3) accumulation of secondary metabolites, including bioactive compounds used as drugs, produced originally by the medicinal plants [26]. These findings have important practical implications for obtaining and producing drugs with improved quality and higher quantity.

CONCLUSION

In conclusion, endophytic fungi isolated from plants and vegetables have been shown to confer some beneficiary and pathogenic effects as enumarated on this research. More researches are required to be done on the benefits and pathogenesis of fungi isolated from plants and vegetables.

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