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Review

Plant Growth Promoting Filamentous Fungi and Their Application in the Fertilization of Pastures for Animal Consumption

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Abstract: The diversity of fungi in different terrestrial and aquatic ecosystems has made it possible to explore their use as important tools in promoting plant growth and in managing plant diseases given their high potential to replace the use of synthetic chemical products (fertilizers and pesticides). Therefore, this review compiles information on the use of filamentous fungi in promoting plant growth, highlighting the most studied fungal genera for this purpose, such as *Trichoderma*, *Penicillum*, and *Aspergillus*. In addition, information is compiled on the promotion of forage grass growth using filamentous fungi, which could be a sustainable and lower-cost alternative in producing pastures to help raise animals.

Keywords: plants; forage; phytohormones; cattle raising; fungus

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1. Introduction

Fungi are a diverse group of heterotrophic eukaryotic organisms characterized by the absence of phagotrophy and the presence of a cell wall made of cellulose, chitin, or both [1,2]. These organisms have a nucleus, lack chlorophyll (therefore they are not photosynthetic), reproduce sexually or asexually (by spores), and have branched, filamentous somatic structures [3–5]. There are microscopic fungi (mycelial fungi or molds) that cannot be seen with the naked eye and macroscopic fungi (macrofungi) that can be easily seen; they can be made up of one cell (unicellular) or many cells (multicellular) [6,7]. Armed with their morphological traits and extremely high metabolic diversity, fungi have conquered numerous ecological niches and formed a whole world of interactions with other living organisms [8,9]. Fungal habitats include soil, water, and extreme environments, and with around 120,000 species of fungi already described, it is estimated that there are 2.2 to 3.8 million species of fungi on our planet [10]. Fungi play irreplaceable roles in the functioning of the ecosystem, contributing to the decomposition of organic matter and participating in biological cycles [5,11]. Fungi can adopt different lifestyles, for example, saprotrophs, symbionts, neutrals, or parasites; some species are cosmopolitan, and others, due to their ecological plasticity, can adapt to hostile environments [12,13]. They present various pre-adaptations, including asexuality, the synthesis of pigments such as melanin, and flexible morphologies, which facilitate their persistence and adaptation to extreme environmental conditions [14]. Furthermore, under extreme environmental conditions and low competition, fungi focus on developing abilities that allow them to exploit the natural or xenobiotic resources available under the constraints to which they are exposed [15].

These organisms have positive or negative impacts on all existing life forms and, therefore, on all ecosystems. The main ecological relationships of fungi are with other fungi, protozoa, animals, and plants [8]. Within these ecological relationships, fungi have been responsible for diseases in plants and animals, constituting a constant challenge in the areas of research, diagnosis, treatment, and control [16]. However, the great diversity of fungi has also allowed the exploration of their use as tools in the management of plant diseases (fungal biocontrol agents) given their high potential to replace the use of synthetic chemical products [17]. Additionally, fungi show remarkable metabolic characteristics due to a sophisticated genomic network and are important for the production of biotechnological compounds that have a great impact on our society in many ways (products useful in medicine and industry: vitamins, plasma substitutes, anticancer agents, healing accelerators, secondary metabolites, enzymes, proteins, polysaccharides, and organic acids) [4,18,19].

In the case of plant growth promotion, the filamentous fungi of the genera *Trichoderma*, *Penicillium*, and *Aspergillus* have been extensively studied, since they can colonize and interact with plant roots through several mechanisms that enhance plant growth through nutrient absorption, phytohormone synthesis, induction of systemic resistance, and tolerance to abiotic stress [20–22]. In addition, as biological control agents, they protect plants against the attack of pathogens by competing for nutrients and space and inhibiting the growth of phytopathogens through the production of antibiotics and hydrolytic enzymes [21,23,24]. The strains of the genera *Trichoderma*, *Penicillium*, and *Aspergillus* have been used in various crops, such as tomato, corn, beans, soybean, and lettuce, to mention a few [25–30]. These fungi are ubiquitous and widely distributed in the soil, so there is little risk of introducing new unknown strains [31–33]. In addition, they are easy to isolate from the soil, are stable in various environments, and easily adapt to changing conditions [32,34,35]. In this context, the most important properties of these filamentous fungi that could allow their use in environmentally friendly agricultural practices have been highlighted [36].

However, the selection of appropriate fungal strains for plant growth promotion is extremely important because some strains of the genera *Trichoderma*, *Penicillium*, *Aspergillus*, and *Fusarium* are pathogens of crops and animals [37–39]. Some examples are strains of *T. harzianum* and *T. simmonsii*, which are the causative agents of green mold disease in edible mushrooms [40], while, in the case of strains of the genus *Penicillium*, *P. allii* has been reported as a pathogen of garlic [41], *Penicillium* spp. as a pathogen of citrus fruit, apple, and pear [42,43], *P. expansum* and *P. glabrum* as pathogens of onion, garlic, and *Iris hollandica*, and *P. expansum* as a pathogen of *Tulipa* sp. [44]. In the case of the *Aspergillus* genus, some examples are strains of *A. flavus*, which has been reported as a pathogen of kiwi fruit [45], *A. tubigensis* as a pathogen of cotton [46], *A. flavus* and *A. niger* as phytopathogens of jojoba [47], and *A. aculeatus* as causing bunch rot in grapes [48]. Meanwhile, several species of the genus *Fusarium* have been reported to be the causes of diseases in potato, banana, vanilla, tomato, and acacia, to mention a few examples [49–53]. Regarding pastures, it has been reported that the *Fusarium fujikuroi* species complex live in endophytic association with or cause diseases in these plants [54]. The pathogenic effects of some strains of *Fusarium* on pastures has been reported for Cocksfoot cv. Aberystwyth S 26 and timothy cv. Scots, which were significantly more susceptible to pre-emergence death caused by *F. nivale* and F. *culmorum* than were either perennial ryegrass cv. Gremie or Italian ryegrass cv. RvP. [55].

On the other hand, the use of filamentous fungi to promote the growth of forage pastures is a use that has not attracted attention, even though several species of filamentous fungi naturally form associations with pastures—relationships positive, neutral, and negative [56]. In recent years, the use of filamentous fungi that promote the growth of forage pastures has been considered as an alternative to the use of synthetic fertilizers in the search for sustainable agricultural practices [36,57]. It has been reported that the inoculation of forage pastures with filamentous fungi has helped plants tolerate salinity stress, improved the growth of pastures, and improved the quality of pastures as forage, which benefits animal nutrition [58,59].

2. Interactions of Plants and Fungi

Fungal–plant interactions can be classified as pathogenic, commensalistic, or mutualistic, but in practice few examples fit these descriptions exactly [60]. New molecular methods are providing insights into the dynamics of fungus–plant interactions, showing that fungi change their relationships with plants (transitioning between the trophic states of pathogenesis and symbiosis or between mutualism and parasitism) at different stages in their cycle of life or in response to changing environmental conditions [61–63]. Understanding fungal–plant interactions has important implications for agriculture, including crop rotation, disease control, and risk management [64].

2.1. Pathogenesis

Fungal pathogens can alter a plant's ability to survive, reproduce, compete, grow, or defend itself against herbivores and other parasites [65]. Interactions between plants and pathogenic fungi are determined by plant and fungal genotypes, the dynamic network of interactions within the plant biome, and by ecological conditions [66]. Interactions between plants and phytopathogenic fungi have been viewed from the perspective of pathogenesis and disease [67]. In such interactions, a susceptible host will support fungal growth within its tissues, leading to the development of symptoms and disease. Conversely, non-susceptible (resistant) hosts will block fungal pathogen infection and disease expression [68]. Phytopathogenic fungi invade their hosts through natural openings or wounds, while others penetrate directly the intact plant surface, either by mechanical force or by enzymatic degradation of the cuticle [69,70]. The infection structures of phytopathogenic fungi are specialized, modified hyphae for the invasion of plant tissues; the infection process begins with adhesion to the cuticle and directed growth of the germ tube on the surface of the plant, where appressoria are frequently formed (their formation is induced by specific physical or chemical cues provided by the plant host), the penetrating hyphae accumulating cytoskeletal components at the tips and secreting a variety of enzymes that degrade the cell wall [71–73].

2.2. Commensalism

Commensalisms are interactions between two species in which one benefits and the other experiences no net effect [74,75]. Plant–fungal commensalism refers to the undisturbed existence of fungi within plant tissue which does not affect the host, providing no benefit or support to plant growth in the form of nutrients or secondary metabolites and without causing disease [76]. An example of commensalism between fungi and plants is that reported by Creamer and Baucom [77], where the endophytic fungi *Undifilum* spp. act as commensals for the wild grasses *Astragalus* and *Oxytropis* spp. (crazy grass), giving little benefit to their hosts under certain environmental conditions.

2.3. Mutualism

Plants establish mutualistic interactions, often described as symbiotic, with both prokaryotic and eukaryotic organisms [62]. Fungi are eukaryotic organisms of great importance in the terrestrial evolution of plants and have the ability to develop symbiotic relationships with roots (mycorrhizae). This symbiotic relationship began at the beginning of the Devonian (400 million years ago), when early bryophyte-like land plants entered into endophytic associations resembling vesicular–arbuscular mycorrhizae even before roots evolved [4,78,79]. It has been reported that most plants in natural ecosystems have symbiotic associations with mycorrhizal or endophytic fungi [80]. In addition, it is estimated that 95% of vascular plants have mycorrhizae. Mycorrhizal fungi obtain carbohydrates for their nutrition from plants (up to 20% of the carbon fixed by the plant is transferred to fungi), and fungi serve as root hairs for plants (the hyphae of the fungus branch out in the soil, creating an extensive network of interconnecting hyphae underground), which allows them to absorb phosphorus, nitrogen, and minerals from the soil [81]. If the interaction between a plant and mycorrhizal fungi becomes unbalanced, disease symptoms appear in the host or the fungus is excluded by host-induced defense actions [82,83]. The morphological, phenological, and physiological characteristics of the symbionts influence mycorrhizal functioning at the individual scale, while biotic and abiotic factors mediate mycorrhizal functioning at the rhizosphere, community, and ecosystem scales [84].

While endophytic fungi inhabit host plants at some point in their life and can colonize internal plant tissues without causing significant damage [85,86], it is worth mentioning that endophytic associations differ from mycorrhizae mainly by the absence of a localized interface of specialized hyphae, the absence of a synchronized development between plants and fungi, and the lack of benefits for plants (nutrient transfer) [87]. However, plants may indirectly benefit from endophytic fungi by increased resistance to herbivores, pathogens, or stress, or by other unknown mechanisms [88]. In the case of endophytic fungi, the symbioses with plants are beneficial or neutral and show attributes similar to those presented by the interactions of plants with pathogenic fungi [83]. Fungal endophyte–plant interactions are based on mutual exploitation and the benefits to the organisms involved are rarely symmetrical; some conflicting selection forces are likely to destabilize these relationships [89].

3. Plant Growth Promoting Filamentous Fungi

Plant-associated fungi, called rhizosphere fungi, are also found in the rhizosphere, which use the nutrients released by a host plant and establish plant–soil–rhizospheric fungal interactions that are of great importance for the functioning of ecosystems and environmental sustainability [90]. Fungi resident in the rhizosphere that are useful to plants are called "plant growth promoting fungi" (PGPF) [91]. The associations between plants and fungi that promote plant growth are beneficial for plants, and a wide variety of fungi with these qualities has been observed. According to what has been reported, they are mainly located in the genera *Trichoderma*, *Penicillium*, *Aspergillus*, *Fusarium*, *Mortierella*, *Phoma*, and *Piriformospora* [92–98]. These beneficial fungi have direct and indirect mechanisms that promote growth (in the organs of underground and aerial plants) and plant protection [99]. The mechanisms involved in the promotion of plant growth by fungi include increased access to nutrients by the production of organic acids and siderophores (nitrogen, phosphorus, potassium, zinc, and iron), the production of plant growth regulators (auxins, cytokinins, gibberellins, ethylene, and abscisic acid), the production of hydrolytic enzymes (xylanases, laccases, pectinases, and cellulases), reductions in the amount of ethylene (production of the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD)), increase in water uptake, induction of plant defense mechanisms against pathogens, and relief of different abiotic stresses in harsh environments (fungi induce a reprogramming of gene expression in plants) [100–102]. These mechanisms are manifested in plants in improved germination, seedling vigor, biomass production, root hair development, photosynthetic efficiency, flowering, plant biochemical composition, yield, and control of foliar and radicular pathogens [103].

3.1. *Trichoderma*

Trichoderma is a fungal genus with a cosmopolitan distribution, and its species have been widely reported as promoters of plant growth and as biological control agents, for which they have a high biotechnological value [104]. In addition, their species have shown varying degrees of specificity towards a variety of plant hosts (including monocots and dicots) [105]. Regarding the promotion of plant growth, it has been reported that some strains of *Trichoderma* can colonize and interact with plant roots through various mechanisms that enhance plant growth through the absorption of nutrients and the synthesis of phytohormones (increased biomass of roots and shoots), disease resistance (stimulation of host plant defenses), and tolerance to abiotic stress [106–108]. The promotion of plant growth by *Trichoderma* strains can be affected by various factors, such as the type of crop, growth conditions, inoculum rate, and type of formulation [109]. Meanwhile, *Trichoderma* strains, as biological control agents, protect plants against pathogen attack by competing for nutrients and space and inhibiting the growth of phytopathogens through the production of antibiotics and hydrolytic enzymes [110,111]. The qualities (promotion of plant growth and biological control) that these fungi present have been reported for different groups of plants that include vegetable, herbaceous, ornamental, and forest crops, as shown in Figure 1. Among the *Trichoderma* species most used to promote plant growth are *T. harzianum*, *T. asperellum*, *T. viride*, *T. virens*, *T. longibrachiatum*, *T. agressivum* f. *europaeum*, *T. saturnisporum*, and *T. pseudokoningii* Rifai. In addition, many strains of the genus *Trichoderma* are already commercially available as biopesticides, biofertilizers, and soil amendments; however, it is increasingly common to find mixtures of several *Trichoderma* strains on the market due to their greater consistency in terms of performance [112].

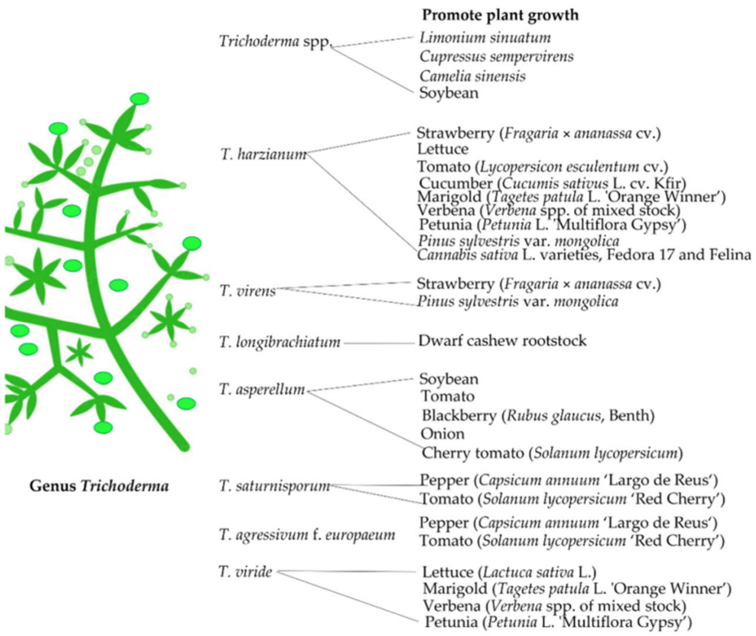

Figure 1. Species of the genus *Trichoderma* used in the promotion of plant growth.

3.2. Penicillium

The genus *Penicillium* includes some species capable of producing gibberellins—substances that can modulate the growth and development of plants [20]. In addition, among the plant growth promoting qualities of some *Penicillium* strains, phosphate solubilization, the production of siderophores, and the production of plant growth regulators have been reported [113]. As some strains of *Penicillium* solubilize phosphate, they have been inoculated in plants that have substrates with phosphorus deficiency, promoting the growth of plants subjected to these conditions [114]. Some *Penicillium* strains have been shown to be growth promoters in plants, such as wheat, sesame, pearl millet, cucumber, sunflower, lentil, soybean, and quinoa (Figure 2).

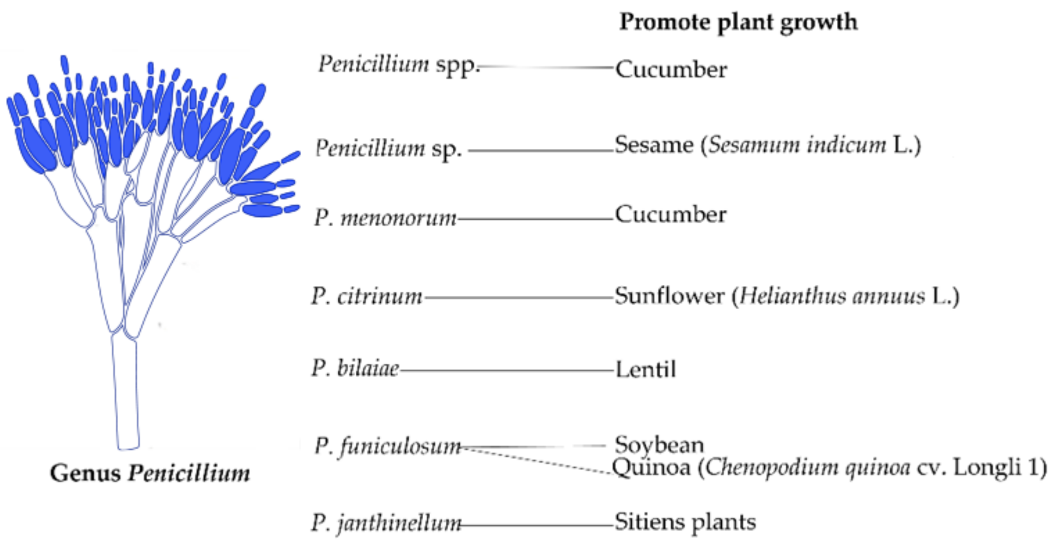

Figure 2. Species of the genus *Penicillium* used in the promotion of plant growth.

3.3. *Aspergillus*

Some species of the *Aspergillus* genus, like other fungal genera, promote plant growth and protect them from phytopathogens [22]. Among the benefits that some strains of *Aspergillus* offer to plants, the extracellular production of phytases has been reported, which implies the mineralization of phosphorus present in inaccessible organic sources, and the secretion of organic acids to make phosphorus available from inorganic sources [115]. Some strains of *Aspergillus* also produce phytohormones, such as auxins, gibberellins, and other secondary metabolites that promote plant growth [116]. Similarly, some *Aspergillus* strains induce systemic resistance and significantly reduce the stress experienced by plants [117]. Among the *Aspergillus* species most used to promote plant growth are *A. terreus*, *A. niger*, *A. awamori*, and *A. japonicus* (Figure 3).

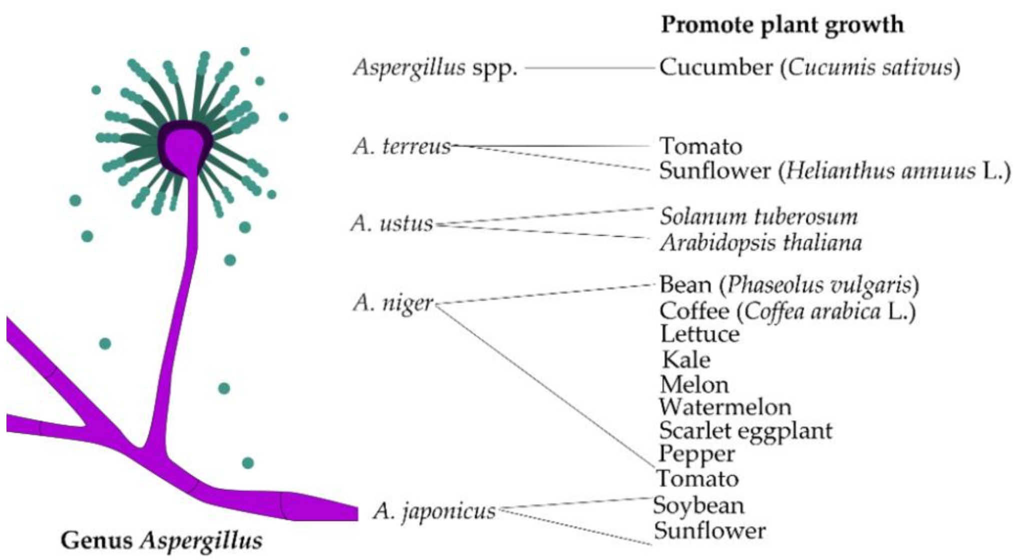

Figure 3. Species of the genus *Aspergillus* used in the promotion of plant growth.

3.4. *Fusarium*

In the case of the *Fusarium* genus, most of the species have been reported as phytopathogens of plants; however, there have been reports that some *Fusarium* strains promote plant growth and protect them against phytopathogens even of the same genus [118,119]. Among the *Fusarium* species most used to promote plant growth are *F. equiseti*, *F. pallidoroseum*, and *F. oxysporum* (Figure 4).

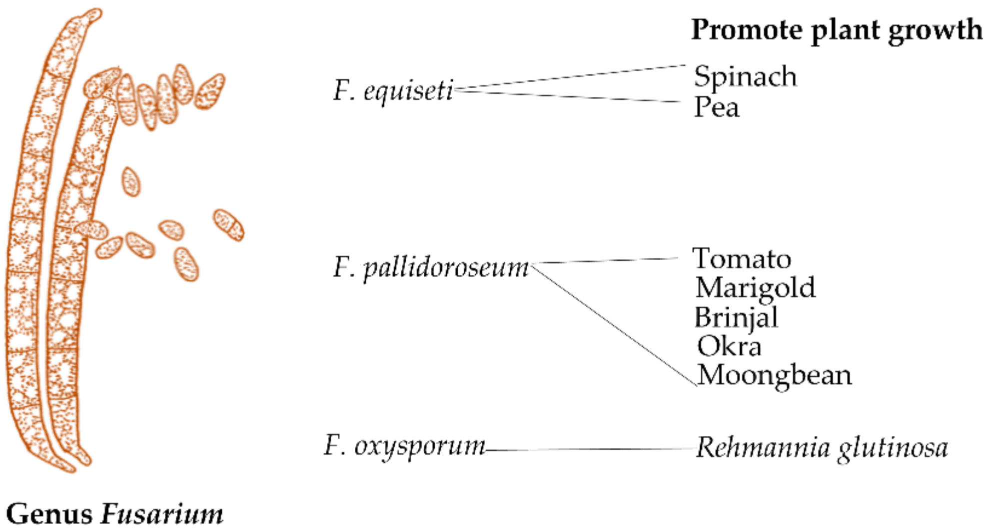

Figure 4. Species of the genus *Fusarium* used in the promotion of plant growth.

3.5. Other Fungal Genera

Other fungal genera for which plant growth promoting strains have been reported are *Mortierella*, *Phoma*, *Piriformospora*, *Purpureocillium*, and *Metarhizium* (Figure 5). Fungal strains that produce phytohormones, such as auxins, gibberellins, and volatile organic compounds, promote the growth of host plants [120,121]. These fungi improve the availability of nutrients, such as phosphorous, nitrogen, potassium, zinc, and iron, to host plants [122]. In addition, these fungi induce systemic resistance in plants and protect them against phytopathogens [123,124].

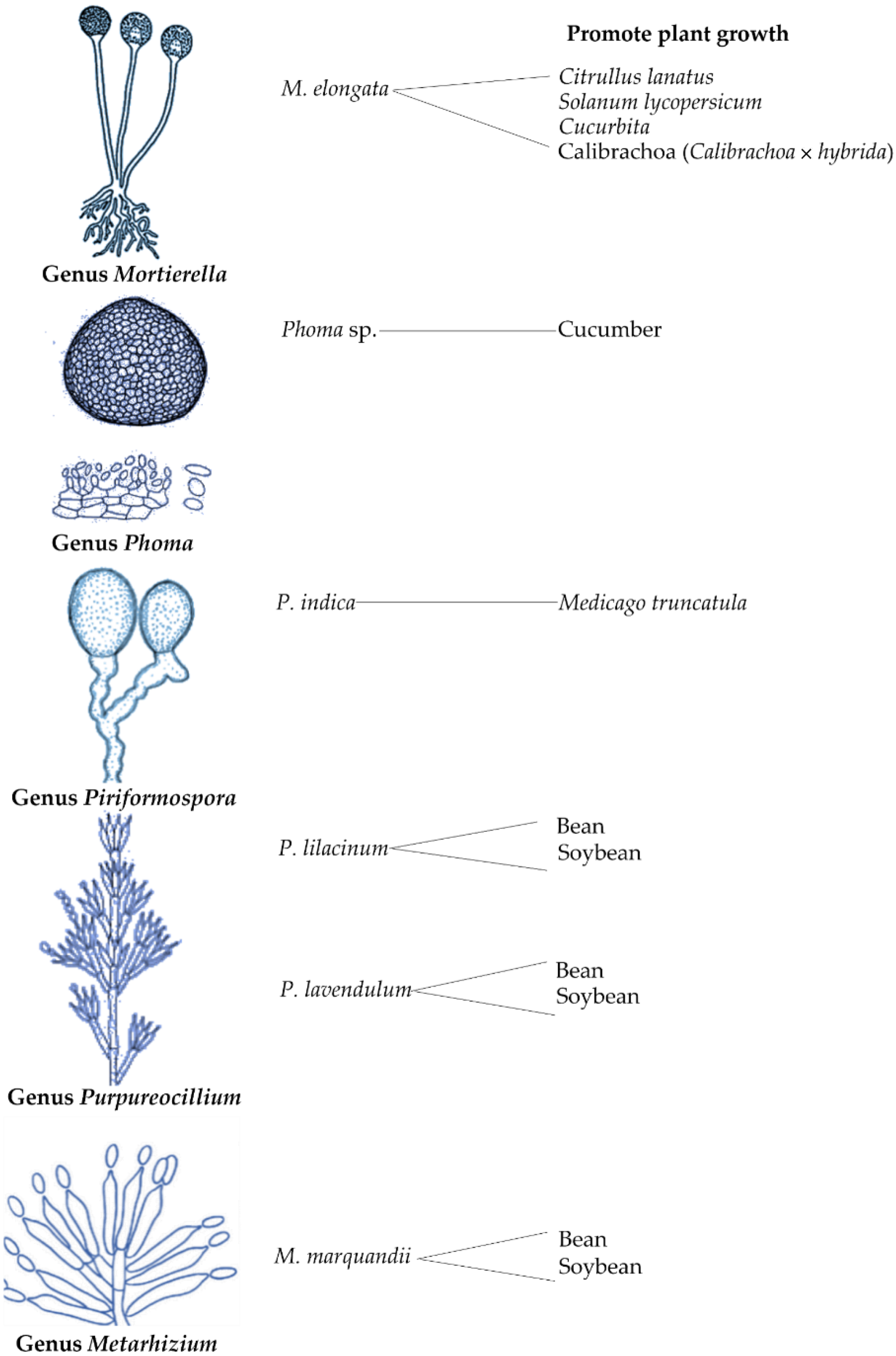

Figure 5. Species of the genera *Purpureocillium*, *Metarhizium*, *Mortierella*, *Phoma*, and *Piriformospora* used in the promotion of plant growth.

3.6. Filamentous Fungi That Promote Plant Growth of Gramineae

The grass family includes about 10,000 species, among which are included economically important species, such as wheat, corn, rice, barley, oats, rye, common millet, African millet, teff, cane sugar, and sorghum, among others [125]. Regarding the promotion of plant growth of grasses by filamentous fungi, it has been reported that strains of the genera *Trichoderma*, *Penicillium*, *Aspergillus*, *Fusarium*, *Purpureocillium*, *Metarhizium*, *Mortierella*, and *Phoma* promoted plant growth and improved the availability of phosphorus for maize, wheat, and rice crops mainly (Table 1).

Table 1. Filamentous fungi that promote the growth of grasses.

Fungal Strains	Grasses	Benefit	References
T. harzianum strain Th3	Wheat (*Triticum aestivum* L.)	*Trichoderma* inoculation significantly increased wheat yield	[126]
T. pseudokoningii Rifai	Rice	*Trichoderma* inoculation increased the availability of phosphorus and zinc, as well as increased crop yield	[127]
P. radicum (sp. nov.)	Wheat (*Triticum aestivum* L.)	*Penicillium* inoculation increased wheat yield in the greenhouse (9%) and in the field (14%), and increased phosphorus uptake (10%)	[128,129]
P. oxalicum	Pearl millet (*Pennisetum glaucum*)	*P. oxalicum* inoculation increased plant growth and nitrogen, potassium, and phosphorus uptake compared to controls under greenhouse conditions	[130]
P. oxalicum I1	Maize	Inoculation with the filamentous fungus increased the corn yield by 14.47% compared to the control	[131]
A. awamori strain Wl1	Maize (*Zea mays*)	*Aspergillus* inoculation promoted the growth of maize plants	[132]
F. pallidoroseum	Maize and wheat	*Fusarium* inoculation improved shoot dry weight and shoot length in all plants	[133]
Purpureocillium lilacinum, *P. lavendulum*, and *Metarhizium marquandii*	Maize	The inoculation of the fungal strains improved the growth of the plants, and some strains increased the availability of phosphorus and nitrogen	[134]
Mortierella elongata	Maize (*Zea mays*)	*Mortierella* inoculation increased height, leaf area, and plant dry weight of *Zea mays*	[96]
Phoma sp. strains GS6-1 and GS7-4	Wheat	The inoculation of the fungal strains promoted plant growth and suppressed root rot caused by phytopathogens (*Gaeumannomyces graminis* var. *tritici* and *Cochliobolus sativus*)	[123]
Phoma sp.	Maize (*Zea mays*)	*Phoma* inoculation promoted plant growth	[135]

4. Importance of Pastures for Animal Consumption

Tropical grasslands are ecosystems in which herbaceous plants, generally grasses and legumes, and woody species, whether shrubs or trees, interact. From the nutritional point of view, it could well be said that grasses, known as pastures or grasses, constitute the main source of nutrients for ruminant consumption. Pastures belong to the family of Poaceae (Gramineae); the word Poa comes from Greek, meaning grasses or forage plants [136]. Poaceae is represented worldwide by 12 subfamilies (Anomochlooideae, Pharoideae, Puelioideae, Oryzoideae, Bambusoideae, Pooideae, Panicoideae, Aristidoideae, Arundinoideae, Micrairoideae, Danthonioideae, and Chloridoideae) with more than 789 genera and about 11,783 species [137,138]. Forage pastures have evolved to resist periodic defoliation and remain vegetative for most of the year [139]. Pastures have two important functions: (1) to provide ground cover to maintain long-term integrity and (2) to provide a source of nutrition for wild and domestic livestock [140]. With regard to the first function, pastures have ecological importance, since they prevent soil erosion, preserving its structure, regulating its fertility, and hosting many beneficial organisms [141], serving as bedding for animals and shelter for crops, livestock, arthropods, reptiles, and earthworms [142].

Regarding the second function, pastures increase profitability per hectare in animal production and guarantee the long-term stability of soil [140]. However, for the second function to be fulfilled, it is necessary to have an adequate understanding of each species and variety of grass, and of the changes in the relationships between yield, digestibility, and protein content during the growth of these plants, this information being essential for deciding which species and varieties of pasture to grow [143]. Pastures have become the main alternative as sources of nutrients for livestock feed [144]. However, to guarantee the sustainability of livestock farming, it is necessary to consider the following aspects: (1) the avoidance of deforestation of new areas to be incorporated into livestock and agricultural activities, (2) the loss of soil fertility due to the excessive extraction of nutrients, and (3) soil erosion [141].

Therefore, the maintenance and productivity of pastures has become a great challenge for the sustainable management of environments throughout the world [145], and the limitations that affect the adequate development of pastures must be addressed. For example, the decrease in the availability of nitrogen, among all nutrients, is considered a limiting factor for pastures, resulting in serious losses for cattle raising [146]. Another important nutritional deficiency is that of phosphorus, an element required by plants and very often a limiting factor in soils, which leads to the production of low-quality pastures [147]. It is therefore necessary to address the problem of soil fertility [148] with the inclusion of microorganisms that promote plant growth, together with phosphorus-solubilizing microorganisms and nitrogen-fixing bacteria, in production programs for pastures intended for animal consumption [149], the use of microorganisms being made a viable alternative to excessive fertilizer use in order to reduce the costs and environmental impacts they cause, such as increasing the risk of soil and water contamination by nitrates [150]. Therefore, it is of great importance to develop agricultural practices that allow the maintenance of the production of forage pastures with greater sustainability. In this context, the use of fungal biofertilizers could be a sustainable alternative [151].

Filamentous Fungi That Promote Plant Growth—A Sustainable Alternative for Pasture Production

Different fungal groups associated with grasses that can act as pathogens, commensalists, and mutualists have been reported for several decades, the genetic factors in each of the two partners showing high variability and a range of effects on plant fitness [56]. Furthermore, the natures of these relationships can be modified by biotic and abiotic factors [152]. Regarding fungi that form associations with grasses, endophytic fungi of leaves, endophytic fungi of roots, and mycorrhizae have been reported [153]. Some of these fungi confer benefits to their grass hosts, such as (1) resistance to grazing herbivores (by promoting a level of jasmonic acid that is antagonistic to the salicylic acid pathway in the plant), (2) resistance to nematodes, (3) resistance to pathogenic fungi, (4) higher photosynthetic rates, (5) drought tolerance, (6) protection against extreme environmental fluctuations caused by climate change, and (7) persistence in the field (Figure 6) [153–156]. Most of the fungi associated with grasses belong to the Ascomycota division. These fungi grow intercellularly and systemically in the aerial parts of grasses, and the asexual symbionts of this division are vertically transmitted to grasses [157]. Among the dominant fungal taxa are *Acremonium*, *Alternaria*, *Cladosporium*, *Epicoccum*, *Beauveria*, *Metarhizium*, and *Penicillium*, which have been reported in many grasses and locations [158,159].

An example of such a positive association is that reported for *Vicia villosa* forage, where, when inoculated by the arbuscular mycorrhizal fungi *Diversisporaspurca*, *Funneliformismosseae*, and *Rhizophagusintraradices* and the endophytic fungus *Serendipitaindica*, it was found that there was an improvement in plant growth performance and root morphology. Furthermore, arbuscular mycorrhizal fungi significantly elevated concentrations of chlorophyll a and b, carotenoids, and total chlorophyll, as well as leaf sucrose, which consequently led to a significantly higher accumulation of glucose and fructose in the roots, providing carbon sources for the symbionts, which positively influenced soil fertility [160]. Another example is the inoculation of white clover (*Trifolium repens*) with an arbuscular mycorrhiza (*Rhizophagus intraradices*) and a rhizobium (*Rhizobium trifolii*), where it was reported that the inoculation with the rhizobium significantly stimulated the colonization of roots by *R. intraradices*, revealing a cooperative interaction between the arbuscular mycorrhiza and the rhizobium which partially magnified the positive effects of nitrogen metabolites (aspartate and proline) and relevant enzymes (asparagines synthase, nitrate reductase, and glutamate synthase) for white clover nitrogen assimilation [161].

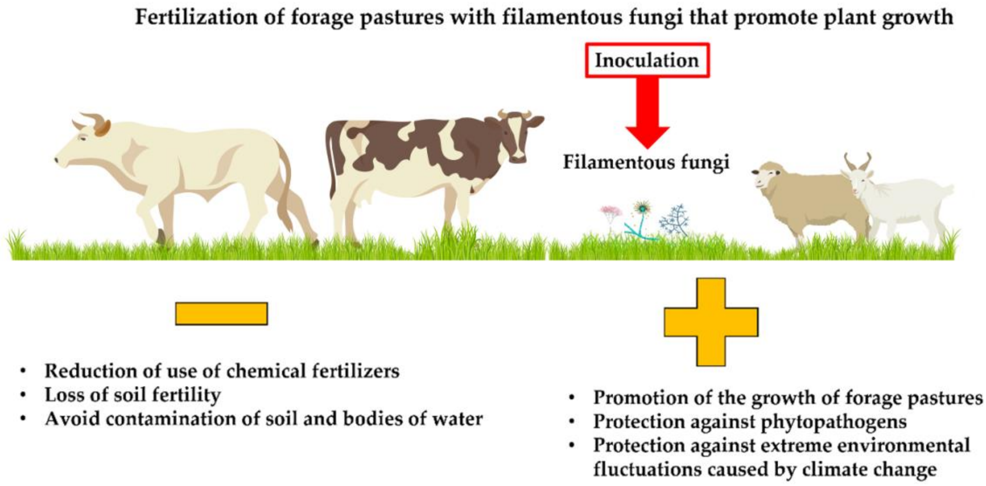

Figure 6. Fertilization of pastures for animal consumption using filamentous fungi.

On the other hand, with respect to inoculation with filamentous fungi to promote the growth of forage pastures, reports are scarce; however, it was found in the studies available that species of the genera *Aspergillus* and *Trichoderma* have been used for this purpose. In the case of the *Aspergillus* genus, it has been reported that inoculation with *Aspergillus aculeatus* helped perennial ryegrass (*Lolium perenne*) plants to tolerate salinity stress; in addition, the inoculated plants showed a higher growth rate and forage quality [58]. Regarding the genus *Trichoderma*, the species *T. harzianum* is the one that predominates in commercial (Trichozam®, Zamorano Biological Control Laboratory and FINTRAC, Tegucigalpa, Honduras and Tricosave®, Labiofam S.A., La Habana, Cuba) and non-commercial inocula [162–165]. The effects of inoculation with *Trichoderma* strains in pastures have been positive in terms of promoting plant growth and control of phytopathogens (Table 2). An inoculum of four strains of *T. atroviride* promoted the growth of perennial ryegrass and controlled diseases caused by *Rhizoctonia solani*, *Sclerotinia trifoliorum*, *Fusarium culmorum*, and *Pythium ultimum* [166]. In addition, *Trichoderma* strains have been inoculated individually or in combination with bacteria and arbuscular mycorrhizal fungi. The combination of *T. harzianum* + *Azospirillum* sp. used to inoculate Marandú grass (*Brachiaria brizantha*) and Guinea grass (*Panicum maximum*) improved the root development and percentage of dry matter of these plants with respect to the controls. However, the best treatment for promoting the growth of these plants was inoculation with *Azospirillum* sp. [165]. This same effect was found for the combination of *T. harzianum* + *Bradyrhizobium* sp. used to inoculate Hybrid Tifton 85 (*Cynodon dactylon*), where the promotion of grass growth due to this combination was better than that due to the control treatments, though it was not the best treatment [164]. When *T. harzianum* (Trichozam®) was combined with vesicular–arbuscular mycorrhizae (Mycoral®, Mycoral Ltda., Cali, Colombia) to promote the growth of hybrid *Brachiaria* cv. *Mulato*, the result was not positive, since the inoculum was associated with the lowest production of dry matter compared to the control treatment [163]—a result that may have been due to the mycoparasitism that some strains of *Trichoderma* present against some mycorrhizal fungi [167].

Although this review has dealt with the role of filamentous fungi on the growth of pastures, it should not be thought that only these plants are susceptible to inoculation with fungal bioformulations, since other species present in grasslands can benefit from rhizospheric fungal activity. The study by Osorio et al. [168] suggests that woody species respond positively to dual inoculation with *Rhizoglomus fasciculatum* (Thaxt.) and *Mortierella* sp., with improvement in the absorption of phosphorus and promotion of the growth of these plants in the nursery, presumably reflecting better development of the plants in the field. As future working hypotheses concerning other important plant species for grasslands, and following the conclusions of Antony et al. [169], it could be said that the conservation of rhizospheric fungi that regulate the growth rates of trees and the development of fungal bioformulations, with the aim of stimulating the growth rates of woody species and regulating nutrient cycles, constitutes an emerging opportunity in the management of the woody components of grasslands and thus the promotion of carbon storage in a changing environment.

Table 2. Species of the genus *Trichoderma* used in the promotion of pasture growth.

Trichoderma	Pastures	Benefit	References
Four strains of *T. atroviride*	Perennial ryegrass	The inoculation increased the dry weight of shoots and roots and controlled four phytopathogens (*Rhizoctonia solani*, *Sclerotinia trifoliorum*, *Fusarium culmorum*, and *Pythium ultimum*)	[166]
T. viride	Raygrass (*Lolium perenne*)	*Trichoderma* inoculation increased green matter and dry matter	[170]
T. harzianum (Trichozam®)	*Brachiaria híbrido* cv. *Mulato*	The inoculation produced the highest dry matter production (145.0 kg/ha/day), but it was not significantly different from the control (131.7 kg/ha/day)	[163]
T. harzianum + *Azospirillum* sp.	Marandú grass (*Brachiaria brizantha*) Guinea grass (*Panicum maximum*)	*Trichoderma* inoculation produced greater root development and a higher percentage of dry matter compared to the control	[165]
T. atroviride	Prairie grass (*Bromus wildenowii* Kunth)	*Trichoderma* inoculation had no effect on seed yield; however, it significantly reduced root infection by *Gaeumannomyces graminis* var. *tritici*	[171]
Mixture of *T. atroviride*	The sterile hybrid grass *Miscanthus* × *giganteus*	*Trichoderma* inoculation increased the chlorophyll content in the leaves as well as the digestibility of the dry material for cattle	[142]
Tricosave® (*T. harzianum*) + *Bradyrhizobium* sp.	Hybrid Tifton 85 (*Cynodon dactylon*)	*Trichoderma* inoculation increased grass biomass compared to the control	[164]
T. harzianum Rifai	*Lolium perenne* L. *Lolium multiflorum* Lam. (perennial ryegrass)	*Trichoderma* improved the growth of both grasses and increased the lengths of the perennial ryegrass leaves	[162]

5. Perspectives and Conclusions

This information indicates that there is a field of study still to be explored, since there are great knowledge gaps regarding the positive interactions between forage pastures and filamentous fungi, which present alternatives to chemical fertilization, which has the disadvantage of contaminating soil and bodies of water, as well as reducing soil fertility. In addition, the price of chemical fertilizers has recently increased due to the war between Russia and Ukraine. In this regard, the filamentous fungi that promote pasture growth could be a sustainable and lower-cost resource for the production of pastures, helping in the raising of animals without affecting soil fertility (Figure 6), several of these filamentous fungi being naturally associated with forage pastures.

However, more in vitro, greenhouse, and field studies are needed to test other fungal genera that promote plant growth on the great diversity of forage grasses used for animal consumption. For these studies, the following recommendations are to be considered:

(1) Use fungal isolates from the rhizospheres of the grasses to be fertilized to produce inocula, looking for fungal strains associated with particular forage grasses.
(2) Use inocula consisting of a single fungal strain, of combinations of several fungal strains, and of combinations of fungal strains with other beneficial microorganisms.
(3) Use different forms of inocula and inoculation.
(4) Consider the efficiency of a fungal inoculum with respect to germination and plant growth.
(5) Determine the effects of external factors on the viability of fungal inocula.
(6) Determine the nutritional contents of forage grasses inoculated with fungal strains.
(7) Consider the production costs of the fungal inocula.
(8) Establish quality standards for fungal bioformulations for forage grasses.
(9) Train farmers in the use of fungal bioformulations for forage grasses.

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