DOI: 10.29050/harranziraat.1524993
Harran Tarım ve Gıda Bilimleri Derg. 2024, 28(4): 550-563

Research Article/Araştırma Makalesi

Antagonistic potential of *Fusarium oxysporum* as an endophyte isolated from Horse-chestnut tree in the management of *Rhizoctonia solani* under *in-vitro conditions*

Rhizoctonia solani'nin mücadelesinde At Kestanesi ağacından izole edilen Fusarium oxysporum'un endofit olarak antagonistik potansiyelinin in-vitro koşullarda belirlenmesi

Muhammad Arif[1*]

[1]Department of Plant Protection, Faculty of Agriculture, Sakarya University of Applied Sciences, Arifiye, 54580, Sakarya, Türkiye.

[1]https://orcid.org/0000-0002-8631-4873

To cite this article:
Arif, M. (2024). Antagonistic potential of F*usarium oxysporum* as an endophyte isolated from horse-chestnut tree in the management of *Rhizoctonia solani* under in-vitro conditions. Harran Tarım ve Gıda Bilimleri Dergisi, 28(4): 550-563

DOI: 10.29050/harranziraat.1524993

Address for Correspondence:
Muhammad Arif
e-mail:
muhammadarif@subu.edu.tr

Received Date:
30.07.2024
Accepted Date:
03.10.2024

ABSTRACT

Symbiotic relationships are established by endophytic microorganisms with their host, resulting in the provision of diverse advantages, such as protection against plant pathogens. Soil-borne pathogens have become a devastating source of infection in many plant species. The environmentally friendly approaches are scare in managing these soil-borne pathogens. This study was aimed to manage one soil-borne pathogen (*Rhizoctonia solani*) by employing another fungal endophyte (*Fusarium oxysporum*) via mean inhibition zone technique. The fungal endophyte was isolated from horse-chestnut tree leaves. The *R. solani* was extracted from the vegetable's plants showing typical symptoms of root rot and damping off. Both endophyte and pathogen were grown on suitable culture media. The antagonistic activity of collected endophyte for soil-borne pathogen was checked via mean inhibition zone technique under in-vitro condition. The diversity indices and isolation frequency analysis revealed that this tree specie has versatile endophytic range. The results from the dual culture experiment assessing the antagonistic activity of endophyte against the soil-borne pathogen (*R. solani*) revealed a significant *(P <0.001)* impact of the endophyte, evaluation times, and the interaction between endophyte and evaluation times on the size of the pathogen colony. The endophyte exhibited a substantial decrease in pathogen development compared to the control, except between days 11 and 15 after inoculation. The data indicate that *F. oxysporum* contains endophytic potential, which might be investigated for potential biocontrol agents against other soil-borne diseases.

Key Words: Chestnut tree; Endophyte; *Fusarium oxysporum*; Diversity indices; *Rhizoctonia solani*; antagonistic potential

ÖZ

Endofitik mikroorganizmalar, konakçılarıyla simbiyotik ilişkiler kurarak bitki patojenlerine karşı koruma gibi çeşitli avantajlar sağlamaktadırlar. Toprak kaynaklı patojenler, birçok bitki türünde yıkıcı bir enfeksiyon kaynağı haline gelmiştir. Ancak, bu toprak kaynaklı patojenlerin mücadelesinde kullanılan çevre dostu yaklaşımlar sınırlıdır. Bu çalışma, fungal endofitik mikroorganizma (*Fusarium oxysporum*) kullanarak toprak kaynaklı patojeni olanı *Rhizoctonia solani*'nın mücadelesi amaçlamıştır. Endofitik mikroorganizma, at kestanesi ağacının yapraklarından izole edilmiştir. *R. solani*, kök

çürüklüğü ve fide devrilmesi belirtileri gösteren sebze bitkilerinden elde edilmiştir. Hem endofitik mikroorganizma hem de patojen uygun kültür ortamlarında yetiştirilmiştir. Toplanan endofitik mikroorganizmanın toprak kaynaklı patojen üzerindeki antagonistik aktivitesi, *in-vitro* koşulları altında ortalama inhibisyon bölgesi tekniği ile kontrol edilmiştir. Çeşitlilik indeksleri ve izolasyon sıklığı analizi, bu ağaç türünün çeşitli bir endofitik yelpazeye sahip olduğunu ortaya koymuştur. Endofitik mikroorganizmaların toprak kaynaklı patojene (*R. solani*) karşı antagonistik aktivitesini değerlendiren çift kültür deneyi, endofitik mikroorganizmanın, değerlendirme zamanlarının ve endofitik mikroorganizma ile değerlendirme zamanları arasındaki etkileşimin patojen koloni boyutu üzerinde önemli *(P <0.001)* bir etkisi olduğunu ortaya koymuştur. Endofitik mikroorganizma, inokülasyondan sonraki 11 ve 15 günler dışında, kontrol grubuna kıyasla patojen gelişiminde önemli bir azalma göstermiştir. Veriler, *F. oxysporum*'un endofitik potansiyele sahip olduğunu ve diğer toprak kaynaklı hastalıklara karşı potansiyel biyolojik mücadele ajanları olarak araştırılabileceğini göstermektedir.

Anahtar Kelimeler: Kestane ağacı; Endofit; *Fusarium oxysporum*; Çeşitlilik indeksleri; *Rhizoctonia solani*; antagonistik potansiyel

Introduction

Endophytes refer to microorganisms, including fungi and bacteria, that reside within the tissues of plants, exhibiting a commensal relationship with the host plant, devoid of any discernible detrimental effects. By establishing symbiotic interactions with their host, these organisms provide a range of benefits, including protection against plant diseases. The importance of these endophytes has increased as they exhibit potential as biological control agents, as they possess the capability to assist plants in combating pathogens and improving overall plant health. There exist several significant mechanisms through which endophytes contribute to the biological control of plant pathogens (Gautam & Avasthi, 2019; Rabiey et al., 2019).

Endophytes have the ability to inhabit distinct ecological niches within plant tissues, thereby impeding the access and establishment of pathogenic microorganisms in these niches. Endophytic microorganisms have the potential to augment the plant's capacity to withstand adverse environmental conditions, including but not limited to drought, salinity, and extreme temperatures (Hardoim et al., 2015; McCully, 2001).Stress enhances a plant's susceptibility to infections, and endophytes' stress-tolerance traits may contribute to disease control . The utilization of endophytes as biological control agents has the potential to decrease reliance on chemical pesticides, thereby fostering the adoption of environmentally conscious and sustainable agricultural practices (Singh & Dubey, 2018; Wani et al., 2015).

The *F. oxysporum* species complex encompasses a diverse range of strains that are widely distributed in soils (Nikitin et al., 2023). The bulk of these strains are saprotrophs, capable of colonizing plant roots. However, they mostly function as commensal endophytes and do not have a significant impact on plant fitness. Certain strains of *F. oxysporum*, such as Fo47 and CS-20, have a positive impact on the host by offering protection against root associated pathogens in multiple plant species (De Lamo & Takken, 2020). Non-pathogenic strains of *F. oxysporum* have considerable promise as biocontrol agents against plant pathogens that dwell in the soil. Through utilizing their capacity to compete against pathogens, stimulate plant resistance, and generate antimicrobial substances, these endophytes provide a natural and sustainable strategy for managing plant diseases (Kour et al., 2008).

About 42-50% of the world's food production is wasted as a result of crop losses imposed on by plant diseases caused by plant pathogens. Food production would have been lost twice if the disease management strategy hadn't been used. Plant diseases are mostly caused by oomycetes and fungi, while some by bacteria, viruses and nematodes (Ezrari et al., 2024; Oraon et al., 2024).

There are several soil-borne phytopathogens which are responsible to destroy the quality and quantity of crops and vegetables. Soil-borne pathogens encompass a range of microorganisms, including bacteria, fungi, nematodes, and viruses, which inhabit the soil environment and have the potential to induce diseases in vegetable plants

(Arif, 2024; Ghazali et al., 2022). The ability of these pathogens to endure and persist in the soil for prolonged durations renders them a substantial source of apprehension for agricultural farmers and horticulturists (Adnan et al., 2017; Arif et al., 2021; Meshram & Adhikari, 2024; Tripathi et al., 2024).

R. solani is one of the devastating soil-borne pathogens that causes adverse effects on a variety of commercially significant crops. There is a significant variation in the appearance of colonies, biochemical and molecular characteristics, ability to cause disease, and aggressiveness among different members of this species. As a result, they have been categorized into 14 groups that are not compatible with each other, also known as anastomosis groups (AGs) (Akber & Fang, 2024; Rafiq et al., 2024).

It is crucial to acknowledge that not all endophytes exhibit beneficial characteristics, and certain endophytes may even exert adverse impacts on their host plants. Consequently, the identification of suitable endophytes for the purpose of biological control necessitates comprehensive investigation and comprehension of their interactions with the particular plant species and pathogens in question. It is imperative to consider various factors such as host specificity, application methods, and the broader plant-microbe ecosystem in order to ensure the successful integration of these practices in agriculture and forestry (Compant et al., 2024; Xiong et al., 2024). The main purpose of this study was isolation and utilization of potential endophyte as biological control agents against one devastating soil-borne pathogen (*R. solani*). This fungal endophyte *F. oxysporum* exhibited the potential to serve as effective biological control agent for the suppression of soil-borne pathogen (*Rhizoctonia solani*). The collected endophytic organism showed the capability to synthesize secondary metabolites that exhibit toxicity towards pathogens, thereby restricting their capacity to infect plants.

Material and Methods

Collection of Specimens

The leaf specimens of Horse-chestnut tree (*Aesculus hippocastanum*) were collected from the garden of the Faculty of Agriculture, Sakarya University of Applied Sciences, 40.698971679765286, 30.34796132350514 and surrounding locations outside the campus, Sapanca 40.71870113026686, 30.252792966279458 district of Sakarya province. To prevent potential contamination with pathogenic microorganisms, the collection process involved gathering healthy and lush green leaves that were disease-free and asymptomatic. All the collected plant specimens were tagged with full information about the plant species, location and coordinates, specimen type, and the date of collection. All the collected leaf specimens were stored in airtight polythene bags to avoid the chances of dying or contamination.

Surface sterilization of specimens

The collected plant specimens were surface sterilized with the help of disinfectants. A series of surface sterilizations was carried out with running tap water to remove the soil debris and other contaminations. The specimens were soaked in distilled water containing a few drops of Tween buffer for 10 minutes. After soaking, these specimens, including roots, stems, and leaves, were cut into desired small pieces. These small pieces were washed twice with sterile distilled water before the surface sterilization. After washing these small pieces with sterile distilled water, these specimens were surface sterilized using 80% ethanol... Later these specimens were treated with 4% sodium hypochlorite and allowed to rinse with sterile distilled water and, at the end, were treated with 70% alcohol for a period of one minute. After treating with alcohol, these specimens were again washed 8-10 times with sterile distilled water and were desiccated under sterile conditions (Sahu et al., 2022).

Preparation of suitable media and culturing of

Endophytes

Dormancy of gene clusters is well-known in plant-microbes co-culturing. Under standard laboratory growth conditions, these silent gene clusters encode secondary metabolites that are not expressed or generated. A possible strategy is co-culturing endophytic microorganisms and plants in the same growing medium. After washing these small pieces with ethanol, alcohol, and sodium hypochlorite, these specimens were dehydrated under aseptic conditions to remove the presence of water or disinfectants. The suitable culture media were prepared according to the type of endophyte diversity. The PDA was prepared for growing plant tissue containing endophytes. This media was autoclaved at 121°C for 15 minutes. The pH of the media was adjusted to 5.6 ± 0.2 (Hamzah et al., 2018; Waheeda & Shyam, 2017). The media was poured into petri plates depending upon the size.

Growing of endophytes

All the collected specimens were tested on PDA to check their growth (Hamzah et al., 2018). Two methods, i.e., the spread and pour plate method and the direct plate impression, were used for growing endophytes.

Direct plate method: The small pieces of each sterile specimen were placed in petri plates containing the culture media. The small pieces of tissue were placed on petri plates containing culture media. These petri plates were incubated at 28±2°C for a period of 15 days or until the appearance of visible growth in the form of colonies (Rashid et al., 2012).

Spread and pour plate method: The aseptically processed sterile tissues were homogenized using a sterile mortar and pestle after removing the outer edge portion. A series of dilutions was carried out to get the final solution. The final solution was inoculated onto the petri plates containing media. All the inoculated plates were incubated at 28±2°C for a period of 15 days or until the appearance of visible growth of endophytes in the form of colonies. The viable colonies were picked for further culturing of this potential endophyte (Athira & Anith, 2020).

Identification of endophytes

Following 15 days of inoculation, the growth characteristics of endophytic isolates grown on appropriate substrates were examined, including the kind of mycelium, color of the colonies, and the rate of growth in relation to various carbon sources. The emerging colonies were picked from each plate and purified on other petri plates. The common characteristics of fungal endophytes, including complex spore, hyphae arrangement, and reproductive structures, were examined using an Axio Vision upright microscope manufactured by Carl Zeiss in Germany. The viable spores were counted by applying the one colony on slide with lactophenol. The Fungal endophyte was identified by comparing its morpho-cultural characteristics with data extracted from published literature and online resources (Song et al., 2016; Zakaria & Aziz, 2018). At last, fungal samples were placed for re-culturing again on PDA plates and kept at room temperature for a maximum of three weeks to provide more clarity. The grown endophyte was stored at a suitable temperature for further pathogenicity analysis. Shannon wiener index was used to calculate the diversity index of endophyte from multiples samples of this tree (Safaie et al., 2024).

Isolation of soil-borne pathogens

The soil-borne pathogens were extracted from the vegetable species grown in Arifiye, Sakarya. The cultures of some soil-borne pathogens, including *Rhizoctonia, Fusarium*, and *Pythium*, are already available in plant protection laboratory which were extracted from soil of vegetable fields. The (*R. solani*) was extracted and purified as described in published literature (Arif, 2024). The isolated soil-borne pathogens were identified with the help of manuals or published literature. The most devastating soil-borne phytopathogenic (*R. solani*) was selected for pathogenicity analysis against the collected endophyte.

Single spore isolation of potential endophyte and

pathogen

After the appearance of fungal colonies of *F. oxysporum* and *R. solani*, a colony illustrating the characteristic morphological features was selected. Spores were collected by gently touching the sporulating area of the colony using a sterile needle with inoculation loop. The spores were placed in sterile water on a microscope slide. The spore suspension was diluted to ensure the individual spores were separated (Parsa et al., 2013). By employing a micropipette, a minute quantity (e.g., 10-20 µL) of the diluted spore solution was carefully moved onto a newly prepared PDA plate. The suspension was equally dispersed to achieve proper segregation of individual spores. The plate was placed in a controlled environment with a temperature range of 25-28°C and left undisturbed for a period of 24-48 hours. The spore concentrations (spores/ml), viable spore concentrations (cfu/ml) of both *F. oxysporum* and *R. Solani* were counted by using the Hemocytometer or Neubauer chamber under electron microscope.

Antagonistic activity of endophyte against Rhizoctonia solani

The collected endophyte was tested against (*R. solani*) under in-vitro condition. The selected cultures of both endophyte and pathogen were assessed by the mean inhibition zone technique. The culture of the pathogen was placed in the center of a petri plate via cork borer, and then one culture of endophyte was spread around the inoculated pathogen. The antagonistic activity of endophyte on pathogen was measured by following the method of (Comby et al., 2017; Kara & Soylu, 2022). Following the randomized plot design (CRD), three replications were set up for each application of in vitro biocontrol, and the experiments were performed twice. A one-way ANOVA analysis was performed on the mycelial inhibition values in the petri dishes using the SPSS statistical program (SPSS Statistics 17.0). The differences between the isolates were examined using the Duncan Multiple Range Test *(P≤0.05).* Mean inhibition zone analysis also known as Kirby-Bauer test was calculated by following equation.

$$\text{Mean inhibition zone MI (\%)} = \frac{\text{M}-\text{R}}{\text{M}} X100$$

The inhibition rate is indicated by MI in percent form, the mycelial appearance of the fungal isolate on media is indicated by M, and the mycelial appearance of the fungal isolate with endophyte is indicated by R.

Data Analysis

The mean inhibitory zones between the endophytic fungi and *R. solani* on PDA was examined using the analysis of variance (ANOVA) Procedure in the SAS program (SAS Institute, Cary, NC, USA, Version 8.0, 1999). The mean values of each parameter for different endophytic fungi were compared using Duncan's multiple range test at a significance level of *P = 0.05*. The SAS software UNIVARIATE was employed to evaluate the data on the dry biomass of individual plants. The analysis compared the control treatment with each treatment involving an endophytic fungus. Additionally, the analysis examined the percentages of inhibition of *R. solani* growth caused by the volatile organic compounds (VOCs) produced by *F. oxysporum*. The mean values were compared using the Tukey's t-test, with a significance level of *P < 0.01 or 0.05*.

Results and Discussion

Recently, there has been considerable interest in a group of fungi known as dark septate endophytes (DSE) that live in the roots of plants. This study also focused on same DSE which was obtained from a horse-chestnut tree. The *F. oxysporum* was used to manage another soil-borne pathogen (*R. solani*) under in-vitro conditions. Multiple independent investigations have indicated that a wide range of endophytic *F. oxysporum* strains has the ability to provide biocontrol, suggesting this as common characteristic (Jaber & Alananbeh, 2018).

The horse-chestnut leaves (midrib, lamina and petiole) that were selected for sampling were

divided into squares of 3 centimeters on each side. Its binomial classification was extracted from published data as shown in table 1. At each stage of growth, three leave parts were taken from the selected.

Table 1. Binomial classification of Horse-chestnut from which endophyte was isolated.

Kingdom	***Plantae***
Clade	*Tracheophytes*
Clade	*Angiosperms*
Clade	*Eudicots*
Clade	*Rosids*
Order	*Sapindales*
Genus	*Aesculus*
Species	*A. hippocastanum*

The overall rate of isolation and frequency of diversity indices was measured from each collected sample. The tree samples collected from inside the campus showed a higher diversity and isolation rate for the single endophyte. The 20 samples were collected from inside the campus while 15 from outside the campus. The positive samples from which endophyte was recovered were 13 from inside the campus, while 7 from outside the vegetative area of campus. The degree of isolation/sample was 0.65 for the samples from inside the campus, while 0.45 was from samples collected outside the campus. The diversity indices for both locations were 1.70 and 2.14 as shown in table 2. The positive sample ratio was high in the samples of inside the campus. The *F. oxysporum* was the most common single specie found at both sites in the current investigation. However, this endophyte was only sometimes found in this tree species. Proposing that growth of these endophytes may be hindered by unfavorable environmental conditions or by the presence of more competitive endophytes that have already established a strong presence in the host tissue.

There has been much discussion over the importance of the endophytic community in endophyte/plant interactions. Endophytes engage in interactions and share functional similarities with other essential microbial communities that inhabit plant tissue, such as pathogens, epiphytes, and saprotrophs. Multiple studies have demonstrated that the existence of endophytes within plant tissues can provide specific benefits to the host plant. Fungal endophytes have the ability to influence plant growth and the way plants respond to infections, herbivores, and changes in the environment. Additionally, certain fungal endophytes can enhance the heat tolerance of the plants they inhabit. Fungal endophytes are a varied collection of fungus belonging to the ascomycetous class. They are characterized by their presence in healthy plant tissues without causing any symptoms. These endophytes have been discovered in plants from all plant families (Comby et al., 2017; Imazaki & Kadota, 2015; Larran et al., 2016).

The mature leaves of the chestnut tree were exposed to fungal propagules for a longer period of time, which led to their growth as endophytes. Additionally, mature leaves provide greater amounts of fungal endophytes as they increase the rate of photosynthetic activity. Interestingly, though, some endophytes found in immature leaves are absent or appear less frequently in adult leaves. Endophytic fungi are a varied collection of fungi that colonize plant tissue without causing any symptoms and do so periodically. The commonly observed genera are *Penicillium*, *Alternaria*, *Fusarium, Colletotrichum*, *Aspergillus*, and *Xylaria*. However, the probability of isolation varies depending on factors such as the plant species and genotypes, plant tissue samples, the geographic location of the plant, and the sampling season. Moreover, endophytic strains can be distinguished from non-endophytic strains due to their distinct evolutionary and ecological background. They

engage in saprophytic, communalistic, or mutualistic interactions with their host plant (Galindo-Solís & Fernández, 2022; Rashmi et al., 2019; Schouten, 2019).

Table 2. The overall rates of isolation and diversity Index of endophytic fungi at collected site.

Site	Inside the Campus	Outside the Campus
Total number of samples collected	20	15
No of +ve samples from which endophyte was recovered	13	7
Isolates/sample	0.65	0.45
Diversity index	1.70	2.14

Table 2 displays the overall rate of isolation for the endophyte assemblages and the diversity indices at each site. A notable disparity in endophyte compositions was observed between the two locations *(Chi-square, $p < 0.005$).* The samples obtained from the campus area shown greater overall isolation rates in comparison to places located outside of it. The two sites vary in terms of their floral composition and spatial arrangement. The chestnut tree withing the campus area are surrounded by other native floral species and a proper maintenance is provided every time, while the host sampled is dispersed and bordered by exotic plants outside of the campus area. Because of the host's dispersed dispersion, there is a limited supply of endophytic inoculum, which explains the poor isolation rates seen outside of the campus area. However, outside of the campus area, a higher diversity of endophytes was seen, indicating that endophytes from the surrounding vegetation can also develop in chestnut trees through stomata or other natural openings as shown in tale 2 and 3.

Table 3. The rates of isolation and diversity indices for the lamina, midrib, and petioles of old and young Chestnut tree leaves.

Tree part/tissue		No of collected samples	No of +ve isolates	Degree of isolation	Diversity indices
Mid-rib	**inside the campus**	8	7	0.87	1.14
Petiole		7	3	0.42	2.33
Lamina		5	3	0.60	1.66
Mid-rib	**outside the campus**	8	4	0.50	2.00
Petiole		5	2	0.40	2.5
Lamina		2	1	0.50	2.00

The quantity of isolates found in the vein and inter-vein tissues did not differ significantly ($p > 0.005$). Regardless of leaf age, more isolates were found in the veins than in the laminas as depicted in table 3. *A. hippocastanum* plants grow in the campus area as shadow trees. The leaves that have been colonized the most are those that are closest to the soil, where endophytic fungi can more easily penetrate and colonize due to increased humidity levels. The presence of more endophytic fungus may also be influenced by the closeness of leaves to litter.

Compared to the midrib and/or petiole, the lamina is reported to be less vulnerable to endophytic infection. The preservation and deposition of spores are influenced by the physical characteristics of the leaf. These include the way water behaves when it reaches the leaf and the way runoff and evaporation patterns occur, all of which work in the midrib and petiole's favour. Additionally, the number of stomata present at particular leaf segments influences endophyte colonization via airborne sources. In terms of nutrition, endophytic fungi are supported more in the midrib and petioles because they typically have more vascular bundles than the intervein region. In comparison to the other tissues analysed, the lamina's fungal endophyte diversity was

comparatively lower.

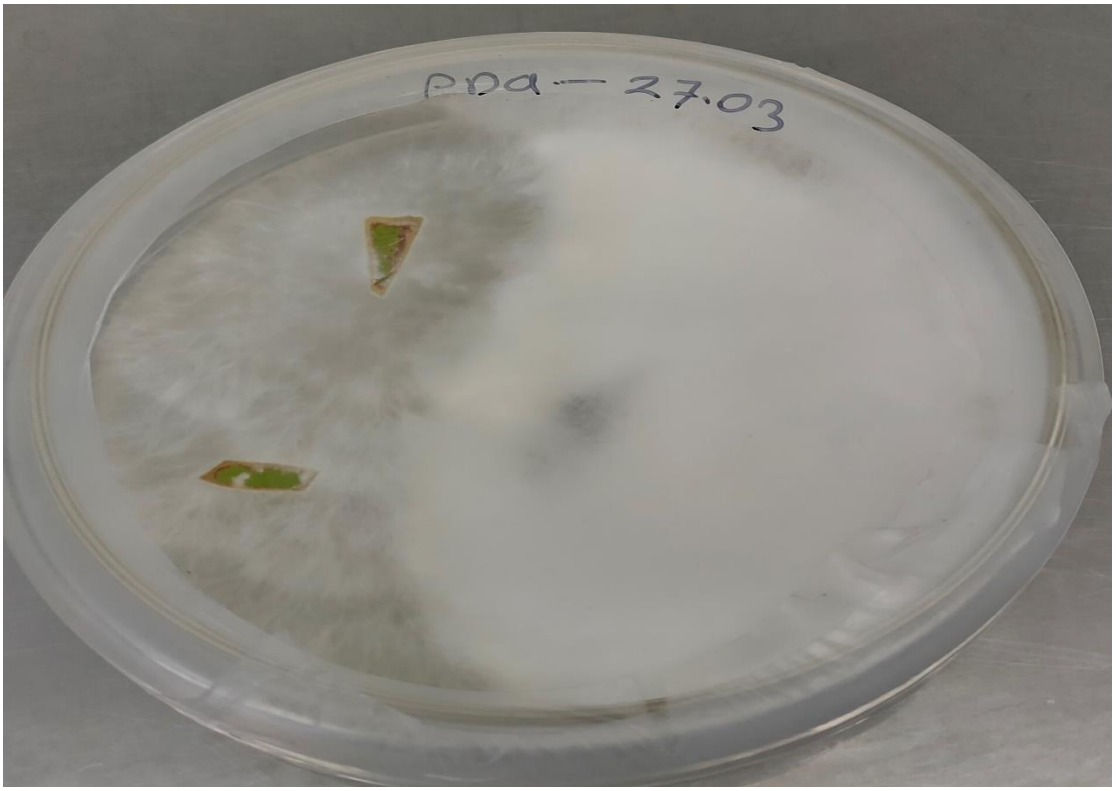

Figure 1. The schematic diagram of colony of *F. oxysporum* on PDA after 2 weeks. The three pieces were placed in each petri-plate for observing the growth of endophytic fungi. Its colony was confirmed by growing and isolating a single spore from each designated petri-plate and its morphological spore structure was observed under microscope.

The endophytic fungi were maintained on PDA at a temperature of 20^0C in the absence of light, until the colonies grew to the edge of the dishes Each fungal isolate was assessed for its colony morphology, which included color and mycelia, as well as the type of spores generated (conidia, blastospores, sporangiospores, or ascospores). This information was utilized to identify the taxonomic classification of each isolate as shown in figure. 1. Pale whitish color was observed after one week of incubation and then start to increase its growth after each day. After 4 weeks of incubation, the incubated plates were removed and stored in refrigerated. The single spore was isolated from the growth of each plate and diluted to check its spore structure and morphology. The diluted culture was saved for further characterization as mycelial inhibitory effect on other devastating soil-borne pathogens. The *R. solani* was selected as phytopathogen to check the efficacy of this endophyte on this soil-borne pathogen. The updated binomial classification was retrieved from the database of soil-borne pathogens as shown in table 4. The fungal hyphae get access to plant roots through many means, such as entering through wounds, fissures in the outer layer of the root, emergence points of lateral roots, or by directly penetrating the tip of the root. The hyphae penetrate the vascular stele by passing through the apoplast of the root cortex. Occasionally, there is evidence of intracellular development accompanied with the demise of the host cell, which is more commonly detected in nonpathogenic strains (Maciá-Vicente et al., 2009; Pérez et al., 2016).

Table 4. Updated taxonomic classification of studied soil-borne pathogen (*R. solani*)

Domain	Eukarya
Kingdom	Fungi
Sub-kingdom	Dikarya
Phylum	*Basidiomycota*
Sub-phylum	*Agaricomycotina*
Class	*Agaricomycetes*
Order	*Cantharellales*
Family	*Ceratobasidiaceae*
Genus	*Rhizoctonia*

The occurrence of an inhibitory zone between endophyte strains and soil-borne pathogens, which corresponds to antibiosis, was identified by dual culture experiments. The fungal colonies were transferred to new PDA dishes, with one colony per dish, and kept at a temperature of 20^0C. The fungal cultures obtained were purified by the process of isolating a single spore or a single hypha tip. Ultimately, the uncontaminated cultures were preserved in a solution of 20% glycerol (volume/volume) at a temperature of -80^0C.

The results from the dual culture experiment assessing the antagonistic activity of endophytes against the soil-borne pathogen (*R. solani*) revealed a significant *(P <0.001)* impact of the endophyte, evaluation times, and the interaction between endophytes and evaluation times on the size of the pathogen colony. The under-investigation endophyte shown a significant decrease in pathogen growth in comparison to the control group within the time span of 7 to 28 days following inoculation. The inhibitory impact was most prominently observed over the initial 3-7 days (Gr1). The growth rates of 2 and 3 were hindered when the pathogen colony reached the border of the petri-dishes after 7 days or halted to expand.

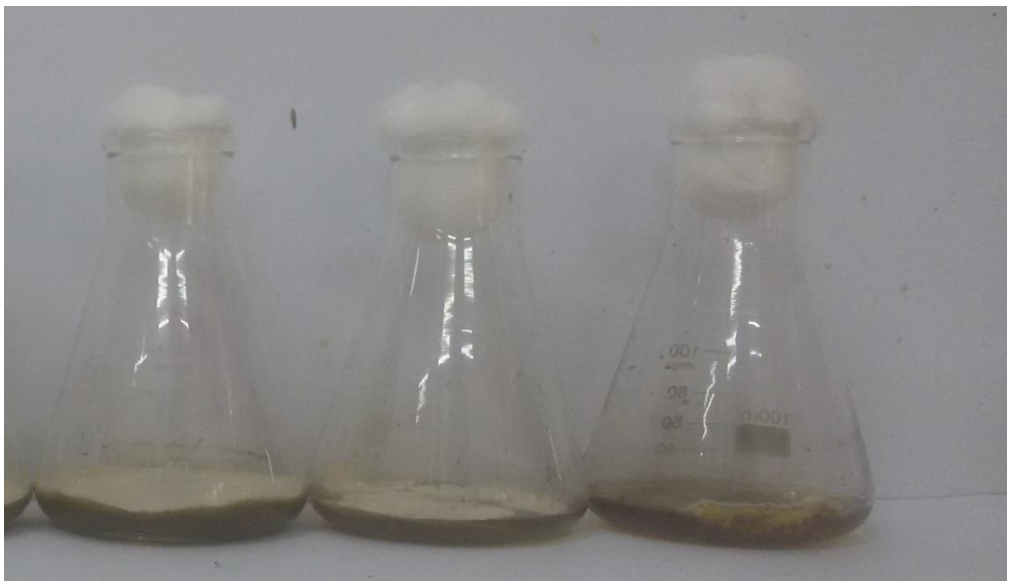

Figure 2. The growth of *R. solani* on PDA media inside the flasks. The colony of pathogen was observed after 7 days. A series of dilutions were carried out to obtain the single spore of this pathogen.

Upon evaluating the colony interaction between endophyte and pathogens on PDA in all possible paired combinations, we noticed many forms of interactions. The figure illustrates 2 the interactions between a pathogen and an endophyte, with the following categories being designated: Situation 1 refers to the growth of two organisms, *R. solani* and *F. oxysporum*., that are mutually blending. Situation 2 refers to a similar growth pattern, but with *F. oxysporum* growing above *R. solani*. Situation 3 describes an instance where *R. solani* ceases to grow and is being

overgrown by *F. oxysporum*. Situation 4 involves mutual inhibition at a distance, where the pathogen is opposed to *F. oxysporum*. The control group consists of the pathogen.

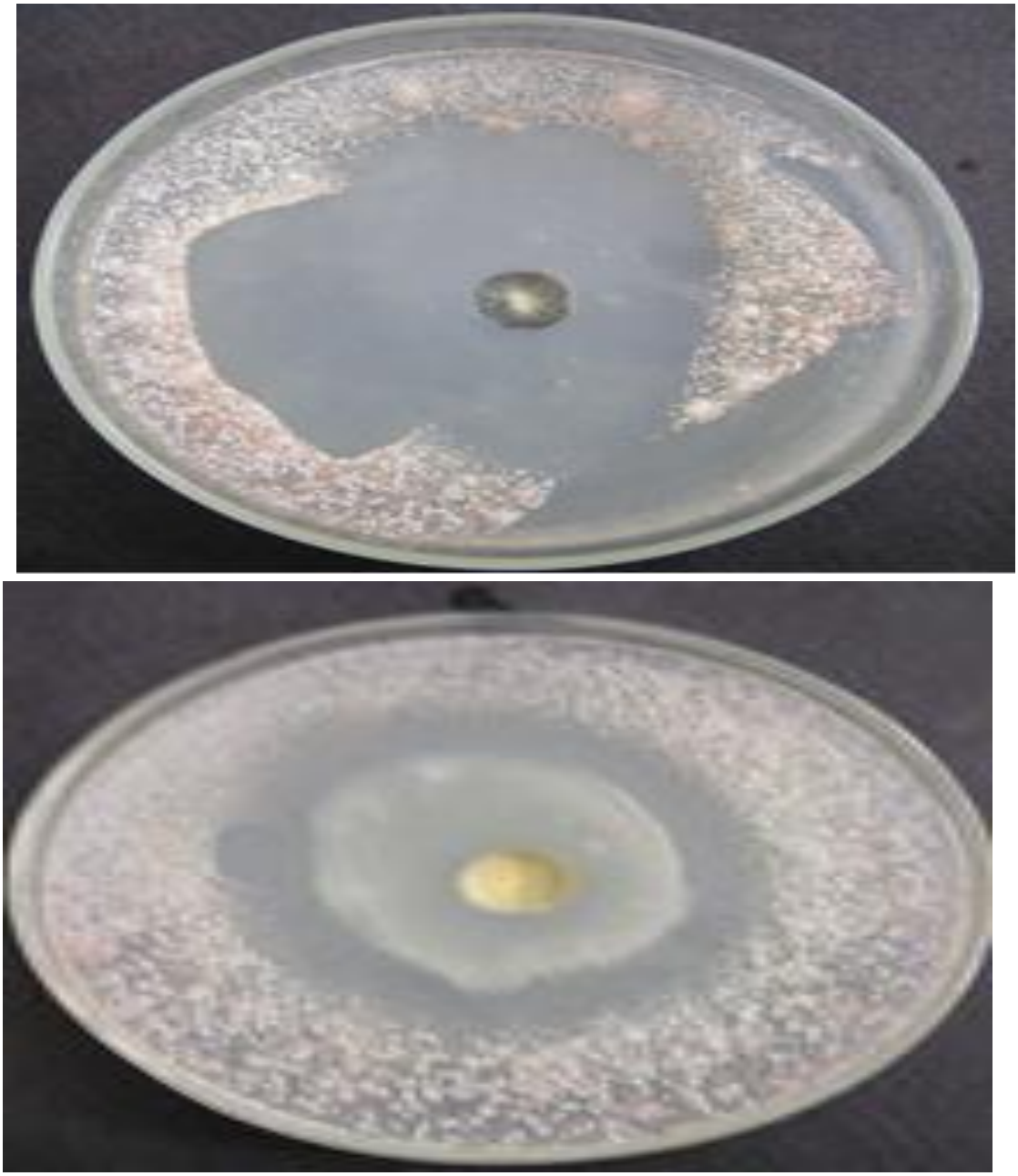

Figure 3. The mean inhibition zone activity of *F. oxysporum* on *R. solani* on PDA medium after 2 weeks. In the middle of the plate, the selected endophyte was paced and other parts of the plates, the soil-borne pathogen was scattered.

The study conducted an analysis of variance (ANOVA) to compare the growth rates of *R. solani* in the presence of the endophyte *F. oxysporum* at four different evaluation times.

Table 5. ANOVA for the Endophytic efficacy of *F. oxysporum* against *R. solani* under in-vitro experimental conditions

Source of variation *(SOV)*	Means squares	Degree of freedom (*DF*)	*F values*	*P values*
Endophyte	142.44	9	*149.2*	*P<0.001*
Experiment	0.270	1	*0.26*	*0.592*
endophyte*experiment	0.452	9	*0.50*	*0.881*
Errors	0.958	55		
time period	157.60	4	*1945.80*	*P<0.001*
time period*experiment	0.091	4	*1.20*	*0.302*
endophyte*time period	9.450	36	*116.50*	*P<0.001*
experiment*endophyte*time period	0.145	36	*2.05*	*0.012*
Errors	0.083	220		

The findings from the dual culture experiment assessing the antagonistic activity of endophytes against *R. solani* indicate a substantial *(P < 0.001)* impact of the endophyte, evaluation times, and the interaction between endophyte and evaluation times on the size of the pathogen colony. With the exception of days 11 through 15 following inoculations, the endophyte showed a significant reduction in pathogen development as compared to the control (Table 5 and 6). According to Table 7, the most effective suppression was observed during the initial 3-7 days (Gr1). The growth rates of 2 and 3 led to decreased values as the pathogen colony reached the border of the petri-dishes after 7 days or ceased to expand. This concept was substantiated by a study in which more than 200 distinct nonpathogenic *F. oxysporum* strains obtained from a tomato field demonstrated the ability to provide biocontrol in tomatoes. Furthermore, it should be noted that the biocontrol method using *F. oxysporum* has proven to be highly efficient in a diverse range of plant species, encompassing both monocotyledonous and dicotyledonous species. *F. oxysporum* has the ability to control certain illnesses caused by oomycetes. *F. oxysporum* (Fo-47) has been documented to decrease the occurrence of diseases caused by *P. oligandrum* in tomatoes, *P. ultimum* in cucumbers, and *P. capsici* in peppers. One shared characteristic of these pathogens is their ability to infect roots. However, unlike other pathogenic *F. oxysporum* strains, not all of them colonize the vasculature (Cucu et al., 2020; Kaur et al., 2011).

Table 6. ANOVA to examine the growth rates of *R. solani* in the presence of endophyte at four different evaluation times in an in-vitro test.

Source of variation *(SOV)*	Means squares	Degree of freedom (*DF*)	*F values*	*P values*
Endophyte	0.839	9	*175.2*	*P<0.001*
Experiment	0.018	1	*0.48*	*0.500*
endophyte*experiment	0.005	9	*1.36*	*0.890*
Errors	0.858	59		
time period	8.66	2	*932.05*	*P<0.001*
time period*experiment	0.018	2	*2.00*	*0.145*
endophyte*time period	0.572	18	*62.80*	*P<0.001*
experiment*endophyte*time period	0.19	18	*2.15*	*0.015*
Errors	0.008	120		

The experiment aimed to assess the endophyte's capacity to hinder spore germination. The findings demonstrate that the chosen endophyte dramatically decreased the percentage of spore germination in the pathogen colony by 80% and 50% in comparison to the control as shown in table 7. Upon conducting microscopic investigations, distinct variations in the morphology of hyphae and conidia were observed between the treatments and the control, in the

pathogen samples obtained from the perimeters of colonies and the paired suspension assay.

Table 7. Means of soil-borne fungal species' (*R. solani*) growth rates and diameters at four assessment intervals during an in-vitro experiment

Experiment	Growth means of *R. solani* (cm)				Days wise growth (cm/day		
	7	14	21	28	1	2	3
Control treatment (without endophyte)	3.05^{d}	8.75^{e}	9.02^{e}	9.06^{e}	1.45^{e}	0.06^{a}	0.01^{b}
F. oxysporum	1.75^{ab}	1.92^{b}	1.92^{b}	1.92^{b}	0.04^{b}	0.01^{b}	0.01^{b}
LSD values 5%	0.34				0.32		
Means	2.12^{b}	2.16^{c}	2.20^{a}	2.25^{b}	2.54^{c}	2.72^{b}	3.02^{b}
LSD values 5%	0.15				0.16		

Means indicates that the observed similarities in letter sharing are not statistically significant according to the LSD Test at a significance level of *P = 0.05*.

Conclusion

In summary, one soil-borne pathogen (*R. solani*) was managed by means of employing another soil-borne pathogen obtained from chestnut tree as endophyte (*F. oxysporum*) by the means of inhibition zone technique under in-vitro conditions. The results of this study will be helpful to manage this soil-borne pathogens via the endophytes as an environment friendly approach. These endophytes can be used as an alternate of fungicidal control of many plant diseases both under in-vivo and in-vitro conditions.

Funding

The work was supported by project number BAP-179-2023, Sakarya University of Applied Sciences, Sakarya, Türkiye.

Author's Contributions

Muhammad Arif was responsible for conceptualization, experimental design's, methodology, analysis, resources, supervision, execution, and writing initial, review and final draft of this manuscript.

Studies in humans and animals

This work does not involve the study of humans and animals.

Conflict of interest

Author declares that no financial or competing interest.

Data availability

All data available within the manuscript.

Consent for publication

Not applicable

Acknowledgment

The author is thankful to the Department of Plant Protection, Faculty of Agriculture, Sakarya University of Applied Sciences for providing lab facilities and SUBU: BAP funding with number (BAP-179-2023) for this work. The author is also thankful to Furkan Doğan for his continuous support in the laboratory.

References

Adnan, M., Zheng, W., Islam, W., Arif, M., Abubakar, Y. S., Wang, Z., & Lu, G. (2017). Carbon catabolite repression in filamentous fungi. *International Journal of Molecular Sciences*, *19*(1), 48.

Akber, M. A., & Fang, X. (2024). Research Progress on Diseases Caused by the Soil-Borne Fungal Pathogen Rhizoctonia solani in Alfalfa. *Agronomy*, *14*(7), 1483.

Arif, M. (2024). Unraveling the diversity and spatial distribution of Soil-borne Fungal Mycobiomes with response to environmental parameters, cropping schemes and cropping seasons. *Journal of Agricultural Biotechnology*, *5*(1), 19-32.

Arif, M., Fawaz, M. S., Zuan, A. T. K., Shah, R. U., Ullah, R., Elshehawi, A. M., Al-Sadi, A. M., Ullah, M. I., Güldür,

M. E., & Alotaibi, S. S. (2021). The impact of different biochars on Stemphylium leaf blight (SLB) suppression and productivity of onion (Allium cepa L.). *Journal of King Saud University-Science*, *33*(7), 101575.

Athira, S., & Anith, K. (2020). Plant growth promotion and suppression of bacterial wilt incidence in tomato by rhizobacteria, bacterial endophytes and the root endophytic fungus Piriformospora indica. *Indian Phytopathology*, *73*(4), 629-642.

Comby, M., Gacoin, M., Robineau, M., Rabenoelina, F., Ptas, S., Dupont, J., Profizi, C., & Baillieul, F. (2017). Screening of wheat endophytes as biological control agents against Fusarium head blight using two different in vitro tests. *Microbiological Research*, *202*, 11-20.

Compant, S., Cassan, F., Kostić, T., Johnson, L., Brader, G., Trognitz, F., & Sessitsch, A. (2024). Harnessing the plant microbiome for sustainable crop production. *Nature Reviews Microbiology*, 1-15.

Cucu, M. A., Gilardi, G., Pugliese, M., Gullino, M. L., & Garibaldi, A. (2020). An assessment of the modulation of the population dynamics of pathogenic Fusarium oxysporum f. sp. lycopersici in the tomato rhizosphere by means of the application of Bacillus subtilis QST 713, Trichoderma sp. TW2 and two composts. *Biological control*, *142*, 104158.

De Lamo, F. J., & Takken, F. L. (2020). Biocontrol by Fusarium oxysporum using endophyte-mediated resistance. *Frontiers in Plant Science*, *11*, 37.

Ezrari, S., Legrifi, I., Taoussi, M., Khadiri, M., Belabess, Z., & Lahlali, R. (2024). Plant–Pathogen Interactions and Global Food Security. In *Plant Pathogen Interaction* (pp. 11-52). Springer.

Galindo-Solís, J. M., & Fernández, F. J. (2022). Endophytic fungal terpenoids: Natural role and bioactivities. *Microorganisms*, *10*(2), 339.

Gautam, A. K., & Avasthi, S. (2019). Fungal endophytes: potential biocontrol agents in agriculture. In *Role of plant growth promoting microorganisms in sustainable agriculture and nanotechnology* (pp. 241-283). Elsevier.

Ghazali, H. M. Z. U., Akram, S., Fatima, I., Hussain, M., Hameed, A., Arif, M., Ahmed, M. A., Al-Ghamdi, A. A., Elshikh, M. S., & Alrashidi, B. O. O. (2022). Fungi species causing dieback and wilt diseases in shisham [Dalbergia sissoo (Roxb)] and impact of various fungicides on their management. *Journal of King Saud University-Science*, *34*(4), 101970.

Hamzah, T. N. T., Lee, S. Y., Hidayat, A., Terhem, R., Faridah-Hanum, I., & Mohamed, R. (2018). Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, Rhizophora mucronata, and identification of potential antagonists against the soil-borne fungus, Fusarium solani. *Frontiers in microbiology*, *9*, 1707.

Hardoim, P. R., Van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and molecular biology reviews*, *79*(3), 293-320.

Imazaki, I., & Kadota, I. (2015). Molecular phylogeny and diversity of Fusarium endophytes isolated from tomato stems. *FEMS microbiology ecology*, *91*(9), fiv098.

Jaber, L. R., & Alananbeh, K. M. (2018). Fungal entomopathogens as endophytes reduce several species of Fusarium causing crown and root rot in sweet pepper (Capsicum annuum L.). *Biological control*, *126*, 117-126.

Kara, M., & Soylu, S. (2022). Isolation of endophytic bacterial isolates from healthy banana trees and determination of their in vitro antagonistic activities against crown rot disease agent Fusarium verticillioides. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi*, *27*(1), 36-46.

Kaur, R., Kaur, J., & Singh, R. S. (2011). Nonpathogenic Fusarium as a biological control agent. *Plant Pathology Journal*, *9*(3), 79-91.

Kour, A., Shawl, A. S., Rehman, S., Sultan, P., Qazi, P. H., Suden, P., Khajuria, R. K., & Verma, V. (2008). Isolation and identification of an endophytic strain of Fusarium oxysporum producing podophyllotoxin from Juniperus recurva. *World Journal of Microbiology and Biotechnology*, *24*, 1115-1121.

Larran, S., Simon, M. R., Moreno, M. V., Siurana, M. S., & Perelló, A. (2016). Endophytes from wheat as biocontrol agents against tan spot disease. *Biological control*, *92*, 17-23.

Maciá-Vicente, J., Rosso, L., Ciancio, A., Jansson, H. B., & Lopez-Llorca, L. (2009). Colonisation of barley roots by endophytic Fusarium equiseti and Pochonia chlamydosporia: effects on plant growth and disease. *Annals of Applied Biology*, *155*(3), 391-401.

McCully, M. E. (2001). Niches for bacterial endophytes in crop plants: a plant biologist's view. *Functional Plant Biology*, *28*(9), 983-990.

Meshram, S., & Adhikari, T. B. (2024). Microbiome-Mediated Strategies to Manage Major Soil-Borne Diseases of Tomato. *Plants*, *13*(3), 364.

Nikitin, D. A., Ivanova, E. A., Semenov, M. V., Zhelezova, A. D., Ksenofontova, N. A., Tkhakakhova, A. K., & Kholodov, V. A. (2023). Diversity, ecological characteristics and identification of some problematic phytopathogenic Fusarium in soil: a review. *Diversity*, *15*(1), 49.

Oraon, S., Padamini, R., Shahni, Y. S., Das, N., Sinha, D., Sujatha, G., Singh, O. B., & Karanwal, R. (2024). Impact of Emerging Pathogens in Crop Production.

Microbiology Research Journal International, *34*(7), 80-92.

Parsa, S., Ortiz, V., & Vega, F. E. (2013). Establishing fungal entomopathogens as endophytes: towards endophytic biological control. *JoVE (Journal of Visualized Experiments)*(74), e50360.

Pérez, L. I., Gundel, P. E., & Omacini, M. (2016). Can the defensive mutualism between grasses and fungal endophytes protect non-symbiotic neighbours from soil pathogens? *Plant and Soil*, *405*, 289-298.

Rabiey, M., Hailey, L. E., Roy, S. R., Grenz, K., Al-Zadjali, M. A., Barrett, G. A., & Jackson, R. W. (2019). Endophytes vs tree pathogens and pests: can they be used as biological control agents to improve tree health? *European Journal of Plant Pathology*, *155*, 711-729.

Rafiq, M., Shoaib, A., Javaid, A., Perveen, S., Umer, M., Arif, M., & Cheng, C. (2024). Exploration of resistance level against Black Scurf caused by Rhizoctonia solani in different cultivars of potato. *Plant Stress*, *12*, 100476.

Rashid, S., Charles, T. C., & Glick, B. R. (2012). Isolation and characterization of new plant growth-promoting bacterial endophytes. *Applied Soil Ecology*, *61*, 217-224.

Rashmi, M., Kushveer, J., & Sarma, V. (2019). A worldwide list of endophytic fungi with notes on ecology and diversity. *Mycosphere*, *10*(1), 798-1079.

Safaie, N., Salehi, M., Felegari, M., Farhadi, S., Karimzadeh, S., Asadi, S., Yang, J.-L., & Naghavi, M. R. (2024). Culture-based diversity of endophytic fungi of three species of Ferula grown in Iran. *Frontiers in Microbiology*, *15*, 1363158.

Sahu, P. K., Tilgam, J., Mishra, S., Hamid, S., Gupta, A., K, J., Verma, S. K., & Kharwar, R. N. (2022). Surface sterilization for isolation of endophytes: Ensuring what (not) to grow. *Journal of Basic Microbiology*, *62*(6), 647-668.

Schouten, A. (2019). Endophytic fungi: definitions, diversity, distribution and their significance in plant life. In *Endophyte biotechnology: potential for agriculture and pharmacology* (pp. 6-31). CABI Wallingford UK.

Singh, R., & Dubey, A. K. (2018). Diversity and applications of endophytic actinobacteria of plants in special and other ecological niches. *Frontiers in microbiology*, *9*, 1767.

Song, J., Pongnak, W., & Soytong, K. (2016). Isolation and identification of endophytic fungi from 10 species palm trees. *International J Agric Technol*, *12*(2), 349-363.

Tripathi, A., Maurya, S., Pandey, K., & Behera, T. (2024). Global Scenario of Vegetable Fungal Diseases. *Vegetable Science*, *51*, 54-65.

Waheeda, K., & Shyam, K. (2017). Formulation of novel surface sterilization method and culture media for the isolation of endophytic actinomycetes from medicinal plants and its antibacterial activity. *J Plant Pathol Microbiol*, *8*(399), 2.

Wani, Z. A., Ashraf, N., Mohiuddin, T., & Riyaz-Ul-Hassan, S. (2015). Plant-endophyte symbiosis, an ecological perspective. *Applied microbiology and biotechnology*, *99*, 2955-2965.

Xiong, X., Zeng, J., Ning, Q., Liu, H., Bu, Z., Zhang, X., Zeng, J., Zhuo, R., Cui, K., & Qin, Z. (2024). Ferroptosis induction in host rice by endophyte OsiSh-2 is necessary for mutualism and disease resistance in symbiosis. *Nature communications*, *15*(1), 5012.

Zakaria, L., & Aziz, W. N. W. (2018). Molecular identification of endophytic fungi from banana leaves (Musa spp.). *Tropical life sciences research*, *29*(2), 201.