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ISOLATION IDENTIFICATION AND CONTROL OF FUNGAL DISEASE OF ANY FRUIT TREE

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ABSTRACT

Pome fruit postharvest infections are currently mostly managed through pre- and postharvest handling procedures and the use of synthetic fungicides. But the deregistration of potent and widely-used fungicides, the emergence of fungicide-resistant postharvest pathogen strains, the rise of integrated pest management (IPM), and the shift toward organic farming have all heightened the need for the development of alternative control strategies (Russell, 2005). Consumer resistance to chemical residues in food and public worry about synthetic fungicides reinforce this need. Pre-harvest management. The preharvest management of postharvest infections involves multiple fungicide applications. Depending on local laws, different countries may have a different quantity of active components. There are often two to four particular pre-harvest fungicide treatments against post-harvest diseases, which are sprayed right before harvest. Many nations currently forbid the use of active ingredient groups like oxyfluoride and benzimidazole (carbendazim, thiophanate methyl), which have a significant impact on the management of postharvest diseases of apples and pears. There is no published data on pathogenic fungi which cause the post-harvest illnesses associated with local

fruits. Present examination was carried out to investigate of numerous fungal pathogens responsible for the post-harvest, decay and deterioration of commercially important fruits from the Cacher area of Assam.

Keywords: Fungal, Fruit Tree, Cacher area, commercially important fruits,

A. INTRODUCTION

Fruits make crucial nourishment for human beings. The high concentration of various carbohydrates, minerals, vitamins and amino acids also provide a favorable platform for the effective growth and survival of numerous parasitic and saprophytic types of fungi (Fatima et al., 2010). (Fatima et al., 2010). Fruits are highly perishable and sustain an active metabolism during the storage phase. During post-harvest time infections can impact the quality of fruits. Post-harvest deterioration of fruits may take place in any phases viz. storage, transit or trans-shipment, during handling activities required to move the commodity from the grower to the whole sale dealer and the retailer and lastly to consumers. Different sorts of fruits are grown in Barak valley but low output of these native fruits could not afford the need of the consumer since they are particularly prone to fungal diseases due to high moisture content and tropical humid climate. In Assam, actual availability of fruits and vegetables in the market decreases down by 35% to 40% due to post harvest losses (State Agricultura Policy, Assam 2004). (State Agricultura Policy, Assam 2004). There is no published data on pathogenic fungi which cause the post-harvest illnesses associated with local fruits. Present examination was carried out to investigate of numerous fungal pathogens responsible for the post-harvest, decay and deterioration of commercially important fruits from the Cacher area of Assam.

FUNGAL IDENTIFICATION

The Ramularia isolated species were identified using the Nomenclature and Species Banks database's morphological identification descriptions (Robert et al., 2005).

Isolation and identification of fungi

Mycorrhizal fungi are isolated using two different techniques. First, soil samples are taken from the host plants' rhizosphere and sieved through a range of screen sizes while being suspended in water. Under a microscope, fungal infection of the roots was seen, and the soil suspension was checked for spores13–15. As an inoculant for the isolation of fungi, soil samples were serially diluted with sterile distilled water at different concentrations (10-1 to 10-7) in the second procedure. Different growing media, including mycological broth agar, potato dextrose agar, Samourai dextrose agar, Capek's dox agar, and malt extract agar, can be used to isolate them. 2,4,5. To extract fungus from environmental materials, potato dextrose agar medium is preferred by the majority of studies6,16–18. Compared to mycorrhizal fungi, endophytic fungi require a slightly different approach to isolation.

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II. CONTROL AND MANAGEMENT OF POSTHARVEST DISEASE

Pome fruit postharvest infections are currently mostly managed through pre- and postharvest handling procedures and the use of synthetic fungicides. But the deregistration of potent and widely-used fungicides, the emergence of fungicide-resistant postharvest pathogen strains, the rise of integrated pest management (IPM), and the shift toward organic farming have all heightened the need for the development of alternative control strategies (Russell, 2005). Consumer resistance to chemical residues in food and public worry about synthetic fungicides reinforce this need. Pre-harvest management The preharvest management of postharvest infections involves multiple fungicide applications.

Depending on local laws, different countries may have a different quantity of active components. There are often two to four particular pre-harvest fungicide treatments against post-harvest diseases, which are sprayed right before harvest. Many nations currently forbid the use of active ingredient groups like oxyfluoride and benzimidazole (carbendazim, thiophanate methyl), which have a significant impact on the management of postharvest diseases of apples and pears. Postharvest management To prevent mechanical wounds that could serve as entry points for wound infections, care must be taken during harvest and postharvest handling. In order to prevent fruit losses in the postharvest phase, alternative techniques to pre- and postharvest fungicide treatments have been investigated. These techniques include the use of biological control agents (BCAs), the application of natural biocides, the induction of natural defense mechanisms of harvested products, and genetic resistance (Jianli and Lepore, 2004; Spadaro et al., 2003).

Additionally, in recent years, there has been an increase in the use of heat, ionizing radiation, ultraviolet C radiation, or CO2 in physical treatments to combat postharvest illnesses (Jianli and Lepore, 2004; Tian, 2007). Despite the significant advances made with biological control agents (BCAs), Mari et al. (2007) stated that these are still not frequently used in the postharvest phase. The main negatives are BCAs' poor and erratic performance, the challenge of finding an appropriate formulation, and the challenge of preventing rot brought on by latent infections. The use of plant bioactive compounds has demonstrated that the treatment conditions (concentration, form of application, formulation, exposure time, time of treatment, etc.) should be established in relation to the treatment response of fruit and vegetable as well as the active substance and fungus pathogen. Elicitors displayed fungicidal activity that was correlated with the timing of the treatment and the stage of plant development, and was occasionally incongruent with fungistatic effects exclusively.

EPIDEMIOLOGY AND POPULATION DYNAMICS OF POSTHARVEST DISEASES

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The epidemiology of the wound pathogens Molinia fructi Gena, Penicillium expanse, and Botrytis cinerea is well understood. On the other hand, little is known about the incidence of the various postharvest diseases that are brought on by quiescent infections during long-term storage and their epidemiology. This ignorance is due to a variety of factors: The differentiation of symptoms between the various diseases is not as clear as for the wound pathogens, resulting in limited data on the relative abundance of the various pathogens. I Less attention was paid to several "minor" pathogens as long as multiple broad-spectrum fungicide applications controlled the build-up of pathogen populations in orchards. (ii) The differentiation of symptoms between the different diseases is not as clear as for the wound pathogens. In a variety of environmental samples, such as host tissues, soil, water, and air, quantitative real-time qPCR enables the accurate, reliable, and high throughput quantification of target fungal DNA. This creates new research opportunities for the investigation of diagnosis, inoculum threshold levels, epidemiology, and host-pathogen interactions (Schema et al., 2004). Species-specific TaqMan PCR tests are a strong method for quantifying fungal populations in environmental materials and can provide fresh insights into the dynamics of pathogen population sizes (Senzeni et al., 2014). To comprehend the links between the accumulation of pathogen inoculum on the various substrates throughout time and infection periods for developing fruits in the orchard, research on disease epidemiology is required.

This information will enable estimation of the relative significance of various substrates as sources of inoculum for fruit diseases. Recently, an unique TaqMan PCR approach was created to identify and measure Stemphylium vesication, a pear-pathogenic inoculum, in pear orchards (Kohl et al., 2013). The effectiveness of disease management measures is increased by evaluating inoculum potential in the orchard environment. With sanitation practices like apple scab and brown spot on pears that lower inoculum loads in the orchard, it can be predicted that disease control of postharvest illnesses will improve. This information can be utilized to create targeted sanitation policies (Hob, 2006; Gomez et al., 2007; Lorene et al., 2010). It will be possible to develop strategies to stimulate beneficial microbiome components or to apply beneficial antagonistic strains to the pertinent plant residues with the aim of suppressing pathogen colonization, survival, and sporulation once it is known how microbial colonizers function in competitive substrate colonization (Kohl et al., 2015). (Carissa and Rolland, 2004; Lorene et al., 2006; Rossi and Pat tori, 2009).

A. FUNGAL DISEASES

For pears of various varieties, at least 13 distinct fungal diseases have been documented globally. Table 1 lists an overview of these ailments, the causative fungi, and the pear cultivars for which they have been recorded. Below is a more thorough explanation of various illnesses.

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Blue mold originates primarily from infection of wounds, such as punctures, bruises, and limb rubs, on the fruit. The first symptoms are soft watery brown spots, which undergo rapid enlargement at temperatures between 20 °C and 25 °C The lesions have a very sharp margin between diseased and healthy tissues and decayed tissue can be readily separated from the healthy one, leaving it like a "bowl" (Pierson et al., 1971; Shim et al., 2002). Blue or blue-green spore masses may appear on the decayed area starting at the infection site. Decayed fruit has an earthy musty odor. The presence of blue-green spore masses at the decayed area and the associated musty odor are the positive diagnostic indications of blue mold (Jones and Dinickel, 1990; Shim et al., 2002). Blue mold occurs on all of the cultivars of pears around the world.

B. OPTIMAL CONDITIONS FOR FUNGAL GROWTH IN PEAR FRUIT

Numerous intrinsic or extrinsic factors have a role in the colonization of any fungi that infect fruit products and the development of mycotoxins. The first contamination of fruit after harvest and before storage as well as the kind of contaminant are intrinsic factors. They also consider the pH, water activity, texture, and nutritional value of the fruit. In actuality, pears are an excellent source of nutrients, have a low pH, and have a high-water activity, making them an excellent medium for fungal growth (Wheeler et al., 1991). The storage temperature, relative humidity, and the atmospheric composition present while wrapping and storing the fruits are extrinsic characteristics.

Fruit should be healthy and provide a thriving environment for microbes. Since it is made up of living tissues, invasion necessitates that fungal pathogens get past defenses that the plant has developed to avoid being infected and die. As a result, there is a specialization between the fruit and various mould species, which allows for the overriding of the fruit's defense mechanisms and the subsequent spoiling. Due to their acidity, fruits are primarily protected from spoiling by fungi; as a result, the study of fruit illnesses brought on by fungi is highly valued (Pitt and Hocking, 1997).

Nowadays, pears are frequently marketed in regions of the world far from the farms where they were grown. Consequently, there is a greater requirement to maintain their quality and a desire for a longer shelf life (Labasa and Breen, 1989). The level of fruit rotting by fungus, which in turn affects fruit losses, is reflected in the choice of post-harvest storage settings for pears. Their deterioration is controlled by seven major physical and chemical variables. Table 2 provides a summary of the ideal growth conditions for the fungi covered in the preceding sections, and the significance of these characteristics is explored below.

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Enemies of food production must be eliminated due to the United States' ongoing population expansion. Agriculture and industry are working together to achieve this goal. Numerous organizations are developing effective control strategies to safeguard our crops against fungal infections. "The chemical industry have much contributed the innovative and very powerful insecticides and familicides now employed for pest control in orchards," claims J.R. Magness (11:23). There are experiment stations set up by the US Department of Agriculture all around the country. There are eight of these stations in the state of Washington, with the primary station at Pullman's Washington State University (16:38) 0 Ultimately, the expense of this quest for novel materials and the cost of recently created programmes fall to the individual farmer. According to J. R. Magness (11:23), 11 Particularly in the eastern states, the new materials have not led to fewer sprays and, in many cases, the newer materials are more expensive, increasing the overall cost of the spray programme. The Agricultural Extension Service has a number of bulletins available that detail the costs associated with producing fruit. The costs associated with eradicating specific illnesses or insects are not separately included in the labour and spraying cost estimates. Disease- and insect-related crop losses have not been discussed. Fruit production is portrayed in a static and unreal way by this.

III. PRESENT LOSSES AND COSTS OF FRUIT-INSECT CONTROL

It is challenging to calculate the entire tax that insects have paid to the fruit business in this nation. Numerous data that have been compiled are thought to be conservative and are based on our best sources of information. These figures were compiled with great difficulty because, in addition to the obvious and direct attacks, the general weakening of the trees and plants that the insects feed on, secondary issues that arise after attacks, the enormous costs of investigations, inspections, and control measures, and the implementation of the many laws relating to harmful species—not to mention the annual tax levied on every fruit grower in the form of the cost of sprays—must also be included. The annual loss of the apple crop in this nation owing to the codling moth was estimated by Quittance in 1907 to be $12,000,000, with the additional cost of control increasing the total to $15,000,000 or $16,000,000. According to him, the total annual loss from fruit insects is $66,000,000. Herrick estimates that treatment for the codling moth will cost $4,000,000 annually and spraying for the San Jose scale will cost $10,000,000. Since the gipsy moth was introduced in this nation, the entire cost of fighting it has surpassed $20,000,000, and we are still spending $1,000,000 a year to do so. According to some expert estimates, the codling moth causes an annual loss of $2,375,000 in Illinois and $2,500,000 in New York.

The annual loss caused by the plum curculio is estimated by Quittance to be $8,500,000. According to Snapp ®, the 1920 plum curculio outbreak in Georgia's peach belt cost peach growers $2,000,000. The cost of enforcing plant quarantines across the various States was

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estimated by Felt to be $1,500,000 in 1923. Despite how large these estimates appear to be, they are well thought out and likely understate the actual cost incurred by these pests.

IV. MATERIALS AND METHODS

The approaches that are taken to investigate the patterns of infection and colonization by endophytic fungi are, for the most part, analogous to those that are taken to investigate fungal plant diseases (Sia Ede et al., 2013). During mycobiotic surveys, the host tissues are sampled in a systematic manner, and the spatial and temporal distributions of the fungal colonists that are encountered are described. These distributions are described using methods to determine the patterns of the endophytic distribution according to the host genera and families, habitat types, infection frequencies related to foliage age, host distribution, and temporal and spatial variations of endophyte infections. The efficiency of a sampling strategy for the detection and enumeration of endophytic fungi can be affected by a variety of factors, including the host species, the host-endophyte interactions, the interspecific and intraspecific interactions of endophytes, the tissue types and ages, the geological and habitat distributions, the types of fungal colonization, the culture conditions, and the selective media. In the present investigation, first four distinct species of cherry (Prunus. sp) were chosen because of their horticultural significance and recent attempts for their application in nursery and orchard trials to find candidate rootstocks for quality control and release in Hungary. This was done in order to ensure that the best possible rootstocks were available.

V. RESULT

A total of 9823 tissue segments (inoculate), 3397 inoculate from roots, 3233 inocula from twigs, and 3193 inocula from leaves of all cherry rootstocks were evaluated, and the results showed that 1614, 2530, and 1037 inocula showed fungal endophyte infection in cultures derived from roots, twigs, and leaves, respectively (Table 4.1).

All of the isolates were first identified based on their visual traits, and then they went through a process called single spore isolation, in which a distinct culture of each unique colony was generated and put through phylogenetic testing using molecular techniques.

Table 1displays the general pattern of endophytic fungi that were isolated from tissue samples collected from cherry rootstocks.

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Tissue	Total number of inocula	Number of infected inocula	Colonization rate % (CR)	Total number of fungi isolates	Isolation rate (IR)
Root	3397	1614	47.5	1944	0.57
Twig	3233	2530	78.3	3427	1.06
Leaf	3193	1037	32.5	1216	0.38
Total	9823	5181	52.7	6587	0.67

In terms of the methodology, the biodiversity of endophytic fungi in 11 rootstocks of Prunus sp. was evaluated by a multidimensional analysis regarding host-specificity, temporal changes, and colonization outlines dependent on the harboring tissue. The results were combined to achieve a comprehensive understanding about the fungal endophytes assemblages associated with the plants that were studied.

VI. IDENTIFIED ENDOPHYTIC FUNGI ASSOCIATED WITH PRUNUS SP., ROOTSTOCKS

Regardless of the analytical values that would help to evolve biodiversity and composition of endophyte infection in examined host plants, the results that were derived from the current study provided brand new information about fungal microorganisms that may contribute to host-endophyte symbiosis in cherry rootstocks within the locality where this study took place. This information was found in cherry rootstocks.

Following the single-spore isolation, 6072 of the 6587 isolates recovered from the roots, twigs, and leaves of cherry rootstocks were taxonomically recognized to the level of genus or species, while a total of 519 colonies remained undetermined. The isolates were taken from cherry rootstocks (marked as unknown).

The two species of Alternaria known as Alternaria sp.1 and Alternaria sp.2 had the highest and second highest number of colonies isolated from cherry trees, respectively, out of all the isolated genera. Alternaria sp.1 had a total of 1931 colonies, and Alternaria sp.2 had a total of 1473 colonies. In contrast, Ceratobasidium sp.1 and Ceratobasidium sp.2 were found solely in root samples and had the fewest number of colonies of any of the other isolates (4 and 15 colonies, respectively).

However, A. sp. 1 displayed the highest frequency in root samples, as 605 colonies of this fungus were isolated from the root samples. The lowest number of isolated colonies belonged to Glomerellaacutata (21 colonies), which was only found in twig samples. A. sp.2 made up

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the greatest population of endophytes with 1349 colonies, despite the fact that this particular isolate was not found in the root. A.sp.1, with its 502 isolated colonies, was the most common fungus in leaf samples, whereas Pyronema sp., with its 10 colonies, showed the lowest frequency in this tissue. This finding is comparable to that of the root samples. In addition to Pyronema sp., two other detected isolates, Rosellinia sp. and Xylaria digitata, were only seen in leaf samples. These isolates were found with Pyronema.

DIVERSITY OF IDENTIFIED ENDOPHYTIC FUNGI IN DIFFERENT SAMPLING PERIODS.

Three different seasons were used to collect tissue samples from cherry rootstocks: the first season began in fall 2008 (season I), the second season began in spring 2009 (season II), and the third season began in autumn 2009. (season III). According to the findings, the Prunus mahaleb rootstocks (Bogdány, SL64, SM11/4, Egervár, Korponay, Magyar, CEMANY, and Érdi V) had a greater overall species richness than the other rootstocks. The average number of unique species that could be separated from Prunus mahaleb rootstocks was 12.5 during the first season, 10.1 during the second season, and 10.1 during the third season. While the Magyar rootstock was found to have a composition of endophytic fungi that contained nine different species, SM11/4 was the source of the greatest number of species that were isolated during season I (16 species). In season II, the species richness of Prunus mahaleb rootstocks ranged from ten species (on the SM11/4 rootstock) to thirteen species (on the Korponay, Bogdány, and Érdi V rootstocks), with SM11/4 having the lowest species richness. When compared to other Prunus mahaleb rootstocks, SL64 had the lowest species richness during season III (8 species), while Korponay had the highest species richness (12 species) in same sample period. The most species were isolated from Korponay during season III. It was determined that there was no significant difference in the species richness of Prunus mahaleb rootstocks throughout the three sample periods. Although a total of 12 distinct species were isolated from Prob rootstock (Prunus fruticosa) during the first season, the species richness experienced a statistically significant drop (P 0.05) over the second and third seasons (7 species, and 8 sepcies, respectively). Gisela6 (Prunus cerasus, Prunus canescens) exhibited a species richness that was quite low, and there was no discernible change across any of the three sample times (6 species, 7 species, and 8 species respectively).

The lowest species richness was found in Vadcseresznye (Prunus avium) during season I (three isolated species), however this index increased (P 0.05) in season II (10 species) and in season III (13 species) (7 species). In conclusion, the average species richness was at its peak during season I (11 species), although it had a modest decline during season II (10.7 species) in

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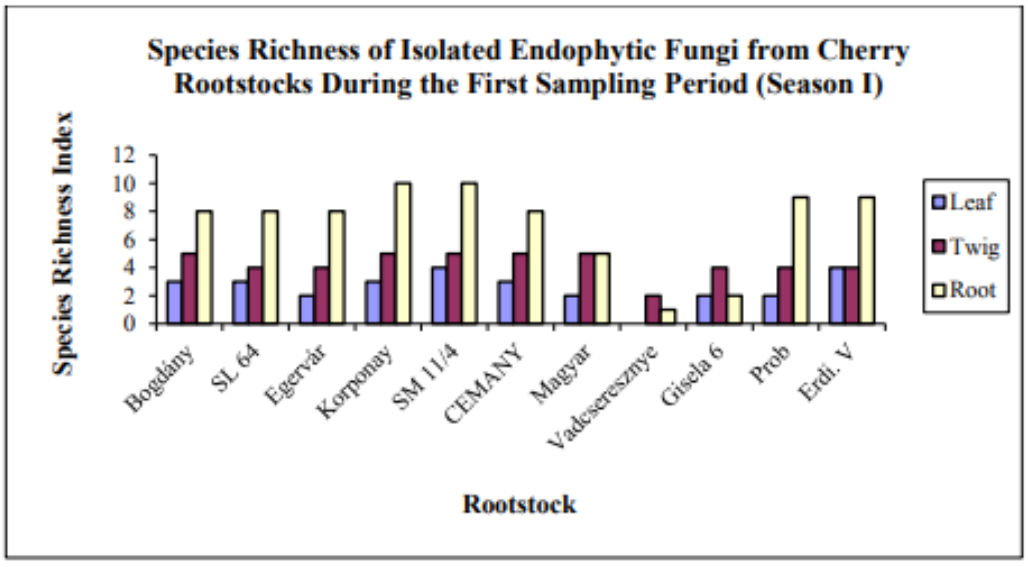
comparison with season III (9.5 species). On the other hand, this difference was not statistically significant. Other rootstocks were associated with an endophytic fungi community that showed comparatively less species richness compared to the endophytic fungi community found in Prunus mahaleb rootstocks, which had the most diverse endophyte communities and almost the same species richness in all four seasons. The diversity of species found in the various tissue compartments of the studied rootstocks is presented in Figure 5.1 for each of the three sample periods. Collectively, root samples had the richest endophytic fungi assemblages in terms of the number of identified species in season I (average 7.1 species, maximum= 10 species, isolated from Korponay and SM11/4 rootstocks, minimum= 1 species, isolated from Vadcseresznye rootstock). This was the case across all three rootstocks tested. The species richness index showed no difference between root and twig as a consequence of an increase in the number of different species isolated from twig samples in this season (average 4.9 species, maximum= 6 species, isolated from Érdi V, Egervár, and SL64 rootstocks, minimum= 3 species, isolated from Gisela6 rootstock). This was a result of an increase in the number of different species isolated from twig samples in this season. During the second season There was a shift in the distribution of different endophyte species during the season III toward a predominant species richness in twigs (average of 6.3, maximum of 8 species isolated from Korponay and Érdi V rootstocks, minimum of 5 species isolated from SL64, CEMANY, Vadcseresznye, and Gisela6 rootstocks). In comparison to the other types of samples, the diversity of species found in leaf samples was the lowest.



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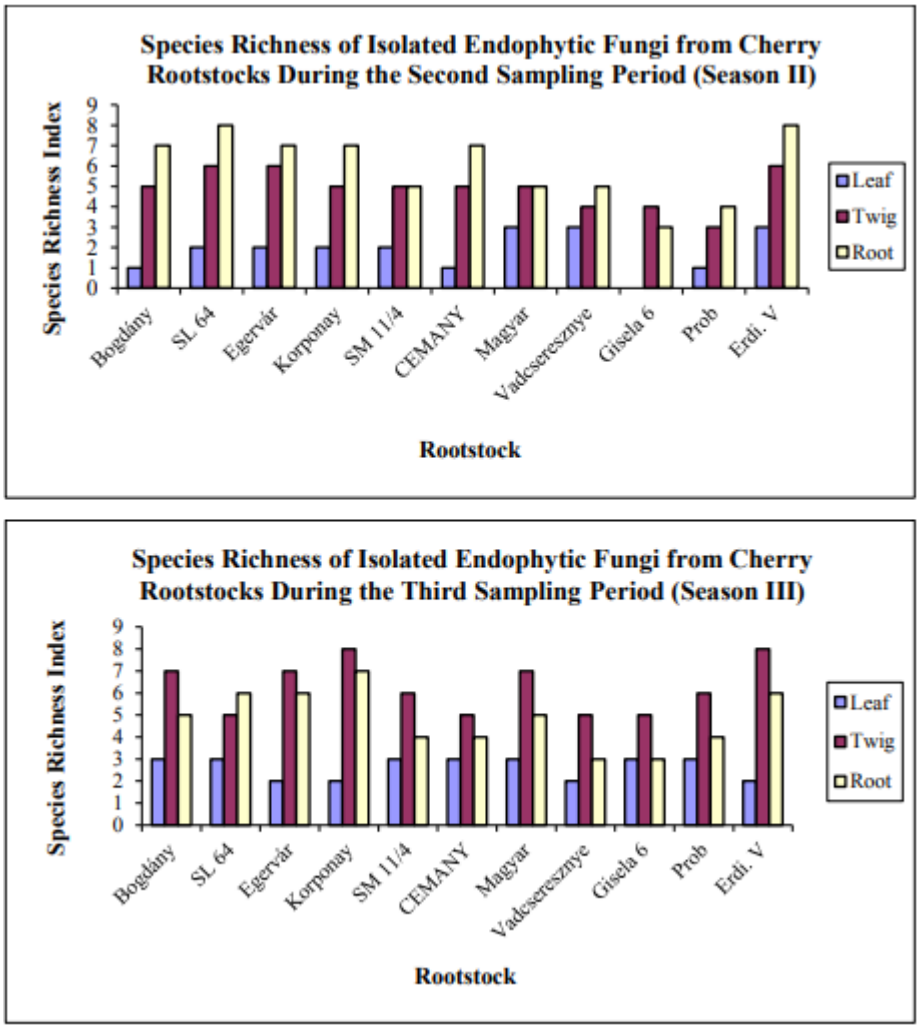

Figure 1: Species richness of endophytic fungi associated with different tissue compartments of examined cherry rootstocks has been demonstrated in three sampling periods.

During season I, the total species richness of leaf samples taken from cherry rootstocks was 2.5 species, with a range that included no isolated fungus in leaf samples taken from Vadcseresznye rootstock all the way up to 4 species in samples taken from SM11/4 and Érdi V. No isolate was obtained from leaf samples of the Gisela6 rootstock in season II (average species richness = 1.8 species from leaves in season II), whereas the Magyar, Vadcseresznye, and Érdi V rootstocks had the highest value of species richness during the second season in leaves (3 species). This was observed in the Magyar, Vadcseresznye, and Érdi V rootstocks. Figure.1 demonstrates that there was no discernible shift in the species richness of leaves during season III in comparison to other seasons; nonetheless, there was a discernible increase in the prevalence of infestations among leaf specimens during this season. The species richness of the leaf during season III averaged out to 2.6 different species, with the largest number of

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species being found on Bogdány, SL64, SM11/4, Magyar, CEMANY, Gisela6, and Prob rootstocks. These rootstocks all had 3 different species. The fewest species were found to have been isolated from leaf samples taken during the season III harvesting of Egervár, Korponay, Érdi V, and Vadcseresznye rootstocks (2 species).

Both the Shannon-Weaver (eH) and Simpson (D) diversity indices were utilized in order to ascertain the range of endophytic fungal species. Not only does the number of species present in a community go into either of these indices, but also the total number of species. The values of eH and D were determined independently for each rootstock based on the total composition of the endophyte communities isolated during each of the three seasons.

CONCLUSION

Endophytic symbionts, which can include bacteria and fungus, are identified in every plant species that has been investigated to this point. These symbionts dwell within the plant's tissues without generating any visible detrimental consequences. It has become abundantly clear that endophytes are rich sources of biologically active natural chemicals that have the potential to be applied in the formulation of medicinal and industrial substances. In addition, research conducted over the past few decades have led researchers to the conclusion that all plants maintain symbiotic relationships with endophytes and epibionts, despite the fact that such a relationship is typically a covert phenomenon in nature. In addition to the xylem of all viable plant organs, fungal endophytes may colonies the tissues of roots, stems, branches, twigs, bark, leaves, petioles, flowers, fruit, and seeds. It is believed that these fungi can alter the ecophysiology of plants. They do this by frequently elevating the host plant's capacity to bear a variety of environmental stresses through mechanisms that are only poorly understood. Endophytes are thought to play key roles in plant protection by increasing the plant's resistance to herbivores, insects, and pathogens. This theory is supported by the observation that endophytes can be found in a wide variety of plant species. In light of this, acquiring an awareness of the pattern of endophytic infection and the diversity of these symbionts in hosts that are found within a particular biogeographical niche is of the utmost relevance for expanding one's knowledge of plant physiology as it currently stands. In addition, any attempt to elaborate on the impact of the plant-endophyte interaction on the biological resistance of host plants against the biological and abiotic stresses that they are subjected to can lead to the implementation of more effective strategies for increasing the quality and quantity of agricultural and horticultural products.

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