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Molecular Characterization of *Botrytis cinerea* Isolates from Different Host Plants

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Abstract:

Background: *Botrytis cinerea* gray mold is one of the most significant diseases that limits strawberry productivity under field and postharvest conditions. It is essential to accurately identify the pathogen and examine potential control options to develop effective disease-management strategies. **Objective:** This study aimed to isolate and identify *Botrytis cinerea* associated with gray mold of strawberry, validate representative isolates through ITS-rDNA sequencing, determine their pathogenicity, and perform an in vitro assessment of the efficacy of selected fungicides, antagonistic microorganisms (AMO) and botanical extracts. **Methodology:** From Dec. 2024 to Mar. 2025, symptomatic strawberry samples were collected from various growing areas of northern Iraq. Isolation of the fungal isolates was performed on potato dextrose agar and subsequently purified with the hyphal-tip technique. Identification was performed first through colony and microscopic characteristics, then confirmed with pathogenicity tests and amplifying the internal

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transcribed spacer region of ribosomal DNA (ITS-rDNA) using primers ITS1 and ITS4. PCR products were sequenced, compared with reference sequences in the NCBI database using BLAST and analyzed phylogenetically using MEGA. In vitro evaluations were conducted to assess the inhibitory effects of selected fungicides, microbial antagonists, and plant extracts. Data were analyzed by analysis of variance and means compared using Tukey's test at $p < 0.05$ when appropriate. **Results:** *B. cinerea* was confirmed in infected strawberry tissues by morphology determination and pathogenicity test. ITS-rDNA sequencing of representative isolates revealed significant homology with reference *B. cinerea* sequences found in GenBank, and phylogenetic analysis confirmed that the tested isolates fell within the *B. cinerea* group. Isolates evaluated differed in virulence. In vitro, all tested fungicides decreased fungal growth, compared to the control with dicarboximide having the lowest EC50 followed by benzimidazole and then other fungicides. Biological Control *Trichoderma spp*. showed the highest antagonistic activity. The botanical extracts tested also inhibited the growth of fungi, and all separated these 12 strains into three antifungal bands; *Allium sativum* had the maximum antifungal effect, followed by *Mentha spicata* and *Tagetes patula*, *Asystasia gangetica* & *Syzygium cumini* & *Rosmarinus officinalis* & *Achillea millefolium*. **Conclusion:** Based on the results obtained, the current study concluded the presence of abnormally shaped colonies through a multi-faceted approach that included pathogenesis tests and ITS-rDNA sequencing to provide accurate identification of gray mold in strawberries (*Botrytis cinerea*). The study demonstrated significant variability among the isolates, highlighting the importance of accurate pathogen identification before implementing control measures. The results indicate that dicarboxymides, *Trichoderma species*, and garlic extract (*Allium sativum*) were the most effective chemical, biological, and phytochemical treatments, respectively. These findings support the feasibility of integrated management approaches that combine biological, phytochemical, and conventional fungicides for controlling gray mold in strawberries.

Keywords: Strawberry gray mold; *Botrytis cinerea*; fungicides; ITS-rDNA; Molecular identification; Plant extracts; Biological control.

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INTRODUCTION

Botrytis cinerea is one of the major economic diseases affecting strawberry production worldwide, known as Actinomycete, which causes a significant reduction in fruit quality and yield. The fungus infects leaves, flowers, fruit, and post-harvest tissues to considerably reduce fruit quality and marketable yield under humid conditions at moderate temperatures. Faba bean is one of the most important legumes that can grow in Iraq. The widespread and highly destructive chocolate leaf spot disease (CLSD), which causes serious damage to faba bean crops, is a well-known and highly tangled threat in the northern regions of the delta area due to low temperatures with high relative humidity. The prevalent low temperatures and high relative humidity in this range promote subsequent spread and severity of the disease, where there have been widely disseminated epidemics [1,2].

Botrytis fabae is usually responsible for chocolate leaf spot, but *Botrytis cinerea* can also be involved in the disease. Many *Botrytis species* that can infect plants at all growth stages have been identified [3]. Epidemiological studies are complicated by the fact that its potential to become latent in host plants makes it difficult to detect early [3,4]. So far, more than 22 species of Botrytis and a hybrid have been identified within this genus.

The classification is based primarily on morphological traits but also on some physiological parameters and the target range. This classification was also supported by some numerical data (2005). The main pathogen *Botrytis cinerea* Pers. Furthermore, it is estimated to infect around 200 different hosts in different environments (e.g., fields, greenhouses, and stores), but without a clear specialization to one specific host [5]. While some species have a wide host range, others are more specific. In contrast, only one or two closely related species within the same plant genus were known to be susceptible, except for *B. fabae*, which can infect many species in the *Fabaceae* family: for instance, *Vicia*, *Lens*, *Pisum*, or *Phaseolus* [5]. However, chemical treatment is complicated due to field isolates of *Botrytis spp*. from mucilage grown on artificial media due to their variance in pathogenicity, morphological characteristics, and fungicide resistance.

This heterogeneity makes it difficult to specifically act on specific strains of *Botrytis*. The high genetic diversity observed is most probably related to mutational and heterokaryosis. On the other hand, the resistant strains appearing in this way provide genetic markers to trace clear genotypic populations of *Botrytis spp*. We have developed a

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powerful polymerase chain reaction (PCR) method for the detection and differentiation of hundreds of diverse pathogenic fungi. PCR amplification of genomic DNA with selected primers can be useful in other instances for identifying plant diseases in untreated materials. [6-8]. Despite many studies explaining the biology and pathogenesis of *Botrytis cinerea*, information is scarce about an integrated approach using morphological diagnosis followed by molecular confirmation, pathogenicity testing, and comparative measurement of chemical, biological, and plant control measures in Iraqi production systems. Hence, efforts should be made towards location-specific management solutions. This current study was conducted to isolate and identify *Botrytis cinerea* (the causal fungus of strawberry gray mold), characterize representative isolates using internal RNA-synthesis (ITS-rDNA) sequencing from smart tools such as Lab Report Free, and also study the effect of some selected fungicides, biologics, and plant extracts on the pathogen under in vitro conditions.

Materials and methods

The research took place between December 2024 and March 2025 in areas where strawberries were ripening. To obtain accurate samples of grey mold illness, farmers in the Duhok region, Erbil, and Sulaymaniyah in Iraq were interviewed. The fields were carefully examined for signs of the illness on the leaves, flowers, and fruits.

Isolation, purification and morphological identification

Between December 2024 and March 2025, a total of fifty deteriorated strawberry samples were gathered from each farming area and transported to the Plant Disease Diagnostic Laboratory at the University of Agriculture to ascertain the presence of the *Botrytis cinerea* fungus. Infected strawberries were sliced thinly (1-2 centimeters) and placed on potato dextrose agar (PDA) for cultivation. Once the pathogenic fungi were identified, they were purified using the hyphal tips technique on PDA medium, and fungal slants were established to continue the research. Utilizing a Nikon AZ100 microscope at magnifications of 10, 40, and 100 times, the germinating spore and fruiting body of the pathogen were observed and documented.[9].

Pathogenicity test

After undergoing sterilization with 70% alcohol, the pristine fruits were then purified with distilled water. Subsequently, the fruit was immersed in a 1% hypochlorite solution for an extended duration, followed by a thorough rinsing in distilled water. A spore

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suspension was introduced into each fruit through the insertion of a sterile needle and subsequent sprinkling of the needle's tip. The inoculated fruit was placed in moist chambers and monitored for 24 hours at ambient temperature. Regular inspections were carried out to detect any signs of disease in the injected strawberries before the onset of the characteristic gray mold. Additionally, further isolation from affected fruits was performed to corroborate Koch's postulates [10].

Molecular identification of grey mold of strawberry

Genomic DNA was extracted and purified from a pathogenic fungus using a modified version of the CTAB method, which proved to be effective. The isolated DNA samples from the pathogen were then subjected to electrophoresis using 1.0% agarose gels in 0.5X TBE buffer with ethidium bromide at a concentration of 100 g/ml. The electrophoresis of the DNA samples from the pathogen was conducted on 1.0% agarose gels in 0.5X TBE buffer, and the resulting DNA bands were visualized and documented using a computerized Gel DocEZ imager. Subsequent analysis involved the amplification of DNA fragments using polymerase chain reaction [11,12].

PCR Amplification

Involves the utilization of Internal Transcribed Spacer (ITS) to manage the process. Primers ITS1 (5TCCGTAGGTGAACCTGCGG3) and ITS4 (5) TCCTCCGCTTATTGATAGC 3) will be employed for the amplification of the internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) of the relevant pathogen. To achieve this goal, a total of 15 distinct isolates were examined. The final step of the Polymerase Chain Reaction (PCR) amplification was conducted using a 15 µl aliquot of green master mix (2X Dream Taq Green PCR), containing 4 mM MgCl2 and 0.4 mM dNTPs (dATP, dCTP, dGTP, and dTTP). Following the addition of 1–10 µl of nuclease-free water to the PCR reaction, the amplification of DNA templates was carried out utilizing forward and reverses (ITS1 and ITS4) primers at concentrations ranging from 0.1–1.0 M.

Sequencing and phylogenetic

The sequence data acquired from the amplified PCR products was utilized to construct a phylogenetic tree in the software MEGA 7.0. Subsequently, the NCBI database was queried using BLAST to locate sequence matches. [13,14].

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Management of grey mold of strawberry

Benzimidazole (1,3-benzodiazole), anilide (aniline), cyprodione (cyprodinil + pyrimethanil), dicarboximide (N-octylbicycloheptene), anilinopyrimidine (cyprodinil + pyrimethanil), triazole (metconazole + propiconazole), and pyrazole are all types of fungicides that were assessed for the management of strawberry grey rot in a study involving Petri dish fungicide evaluation. A detailed analysis of these fungicides, including benzimidazole (1,3-benzodiazole), dicarboximide (N-octylbicycloheptene), anilide (aniline), triazole (metconazole + propiconazole), anilinopyrimidine (cyprodinil + pyrimethanil), and pyrazole, was carried out using the "poisoned food technique" (4-hydroxypyr).

Varied concentrations were used for each fungicide, between 50 g/mL and 250 g/mL. The percentage of inhibition of mycelial growth is calculated using the equation: Inhibition of mycelial growth=100 (Equation 1), where X is the radial growth rate of control plates, and Y is the radial growth rate of plates treated with fungicide. This resulted in 100% inhibition of mycelial growth.

Preparation of plant extracts

Plant components were harvested, subjected to surface sterilization, and subsequently dried in a shaded environment. The materials were then ground to a powder using liquid nitrogen and extracted using 100 % ethanol and methanol in a Soxhlet extractor for 48 hours. All extraction processes were performed as single-solvent extractions at 50 °C under reduced pressure in a rotating evaporator (Heidolph VV2000). Crude extracts were kept at −20 °C for the following study. The extracts were prepared by dissolving them in different concentrations using a stock solution of dimethyl sulfoxide (DMSO) and performing serial dilutions [15]. In these experiments, DMSO functioned as the placebo for the control group. The susceptibility of grey mold pathogens was assessed using the agar well diffusion method, which involved studying the antifungal properties of medicinal herbs such as *Allium sativum*, *Mentha spp*., *Rosmarinus officinalis*, *Achillea millefolium*, *Tagetes patula*, and *Syzygium cumini* [16]. After subjecting the petri dishes to sterilization in an autoclave for fifteen minutes at a pressure of fifteen atmospheres and a temperature of 121 degrees Celsius, twenty milliliters of PDA medium were added to each dish. The autoclave operating conditions were carefully controlled as outlined. Subsequent to the medium

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being set in each petri dish, a sterile cork borer was utilized to create wells measuring six millimeters in diameter in the dishes. Table 1.

Table 1. Medicinal plant species evaluated against *Botrytis cinerea* in the present study

NO.	Ordinary name	Family group	Scientific Name	Part of plant used
1	Jambolan	Myrtaceae	*Syzygium cumini*	Leaves
2	Garlic	Amaryllidaceae	*Allium sativum*	Pods
3	Marygold	Asteraceae	*Tagetes Patula*	Leaves
4	Mint	Lamiaceae	*Mentha spp*	Leaves
5	Yarrow	Asteraceae	*Achillea millefolium*	Leaves
6	Rosemary	Lamiaceae	*Rosmarinu sofficinalis*	Leaves

Various concentrations of solution were prepared in DMSO and pipetted into each well: 5, 15, 25, and 50 g/mL. Each Petri dish housed a single, uncontaminated fungal colony at the center, maintaining a temperature of 25 degrees Celsius. Three separate experiments were conducted as replicates to ensure accuracy. A comparative analysis was conducted by applying a control treatment containing DMSO at the same concentration as the extracts under investigation. The extent of inhibition in radians was calculated using Eq.2. In vitro research has demonstrated the ability of certain bacteria to inhibit the growth of gray mold. The specific pathogenic microorganisms utilized in the study are detailed in the subsequent paragraphs. The comprehensive list of adversaries is provided in Table 2 for reference.

Table 2. Microbial antagonists evaluated against *Botrytis cinerea*

No.	Type	Antagonists
1	Fungi	*Penicillium spp*
2	Fungi	*Colletotrichum gloeosporioides*
3	Fungi	*Trichoderma spp*
4	Fungi	*Aspergillus fumigatus*
5	Bacteria	*Burkholderia cepacian*
6	Bacteria	*Bacillus subtilis*

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Statistical analysis

All experiments were conducted twice, employing a completely randomized design with three independent replicates. None of the experimental conditions yielded a statistically significant interplay between the two evaluations. Subsequently, the outcomes from both trial rounds were combined. The statistical examination was carried out utilizing Statistix® 8.1 software. Data were subjected to analysis of variance ANOVA to determine data significance, with mean distinctions between treatments assessed through Tukey's HSD test ($P < 0.05$).[17].

Results

There were three replicates for each of the three concentrations, seven treatments, and sterile water was utilized as a control in the experiments. The in-vitro studies were conducted using the Poisoned Food Technique. At concentrations of 100, 200, and 300 g/mL, *Alternaria alternata* was the most prevalent fungus (10.09%), followed by *Macrophomina* (4.27%), *Rhizoctonia* (3.49%), *Mucor* (3.21%), *Rhizopus* (1.05%), *Curvularia* (0.79%), and *Aspergillus* (0.98%). The findings are illustrated in figure 1 and 2. figure 1 provides a visual representation of the data from Tables 3 in a stacked bar chart format. Strawberry plants are susceptible to various fungal diseases, with hybrid inoculum sourced from different environments.

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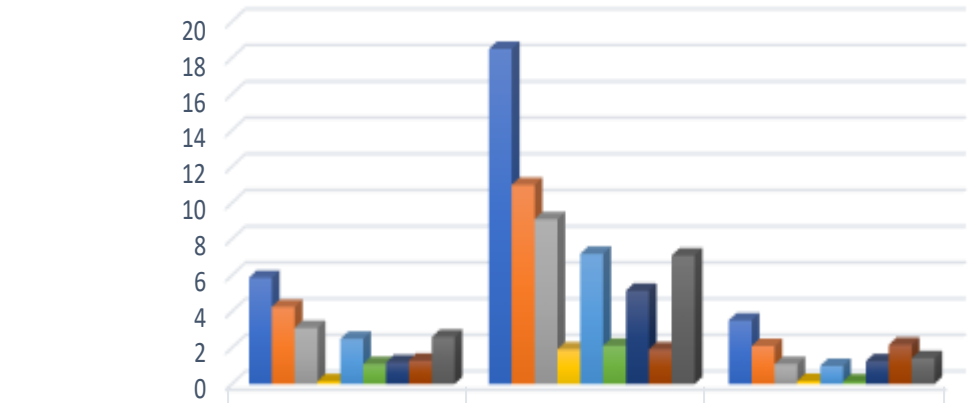

	Leaves	Fruits	Flowers
Botrytis cinerea	5.87	18.5	3.52
Alternaria alternata	4.26	11	2.1
Macrophomina spp	3.11	9.1	1.1
Aspergillus niger	0.16	1.91	0.18
Rhizoctonia spp.	2.5	7.19	1
Curvularia spp.	1.11	2.12	0.14
Mucor spp.	1.21	5.16	1.26
Rhizopus spp.	1.3	1.91	2.17
Means	2.6	7.1	1.43

Figure 1: Frequency (%) of fungal species isolated from different strawberry tissues collected in Duhok Province

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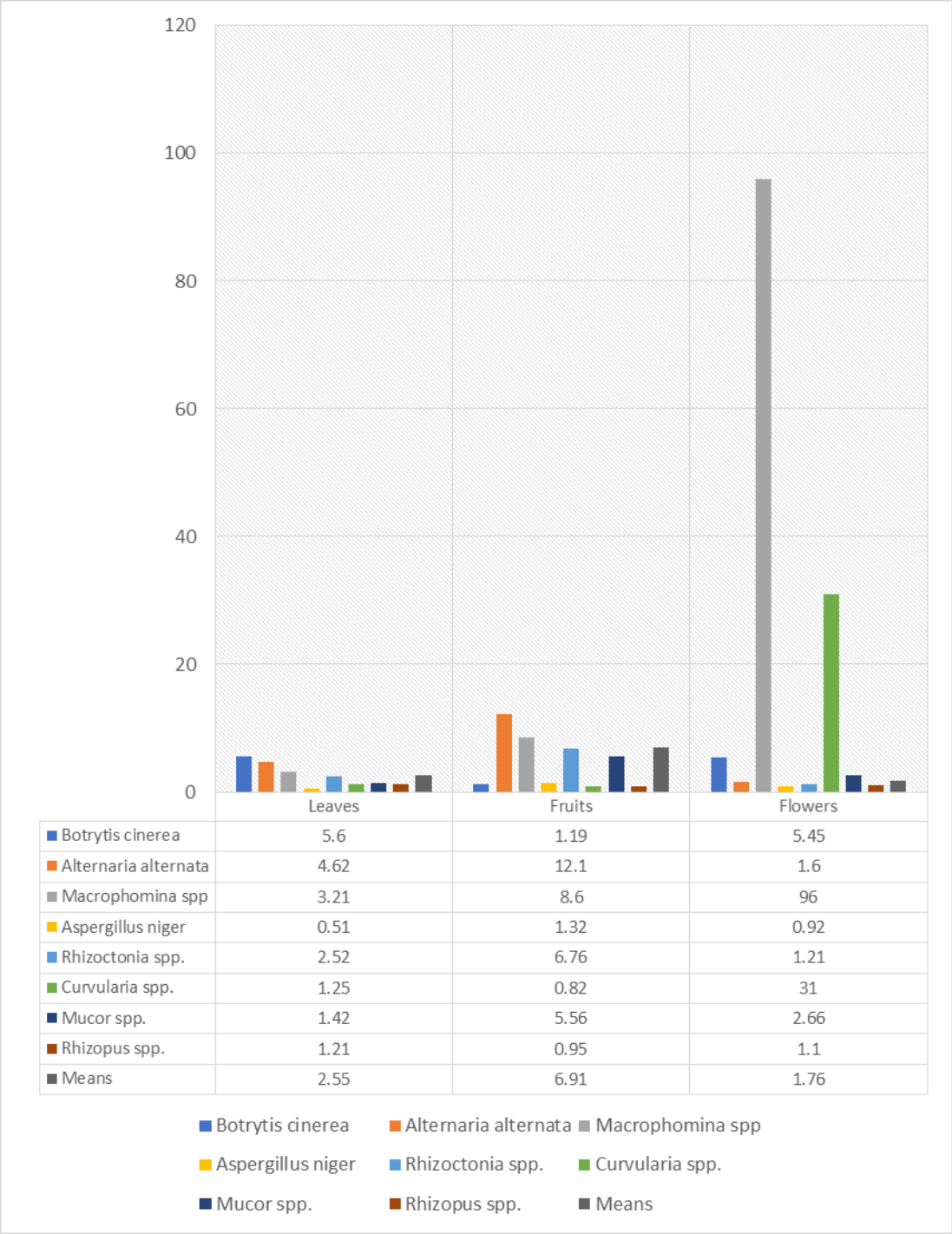

	Leaves	Fruits	Flowers
Botrytis cinerea	5.6	1.19	5.45
Alternaria alternata	4.62	12.1	1.6
Macrophomina spp	3.21	8.6	96
Aspergillus niger	0.51	1.32	0.92
Rhizoctonia spp.	2.52	6.76	1.21
Curvularia spp.	1.25	0.82	31
Mucor spp.	1.42	5.56	2.66
Rhizopus spp.	1.21	0.95	1.1
Means	2.55	6.91	1.76

Figure 2: Frequency (%) of fungal species isolated from strawberry tissues collected in Erbil Province

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A compound microscope was employed to visually confirm the identity of Conidiophores following morphological validation of the culture. At high magnification, a conidiophore bearing egg-shaped, ellipsoidal conidia was observed, indicating the presence of the grey mold pathogen. A greenhouse with healthy plants was used for the experiment, and two populations were performed: a population injected with a concentrated pathogen solution (4 106 CFU/ml) in one group and an uninfected group. On command, no symptoms appeared in the epidermis of healthy fruit throughout experiments: whereas on day seven, signs of soft rot were detected in plant tissues of *B. cinerea* infected group; by 10 days post infection, functional tissues started to collapse rapidly, revealed a marked increases in the infection rate and at fifteen days post-infection fruit-apples were completely disintegrated indicating total takeover of said fruits by *B. cinerea*. In contrast, the control group showed no symptoms of disease and was fully resistant. A decreased crop yield (24 to 25%) was recorded when comparing the infected groups with the controls.Table 3 summarizes the differences in their pathogenicity between Narowal and NARC.

Table 3. Development of gray mold symptoms on strawberry fruits following artificial inoculation with *Botrytis cinerea* isolates during the pathogenicity test.

Day	Infection stage	Control crop	NAROWAL ISOLATE	NARC ISOLATE
7	Visible soft rot	No	Yes	Yes
11	Collapse of parenchyma	No	Yes	Yes
17	Shriveled fruit	No	Yes	Yes
23	Greyish fungal growth	No	Yes	Yes

The main goal of the pathogenicity assays was not to confirm yield loss, but rather to validate detection and identification of a pathogen. According to Gene Analysis, Identification of molecular data and construction of a phylogenetic tree. Based on screening studies for fungal pathogen colonization frequency, it was concluded that amongst the isolates from strawberry, only *Botrytis cinerea* could cause grey mold disease in

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strawberries among the pathogens tested. As such, research focus on this fungus serves well for future investigations.

The ITS region of the 18S rRNA gene from five representative *B. cineria* isolates was selected for genetic analysis, with amplification performed using ITS 1 and ITS 4 primers as outlined in the materials and methods section. Using the maximum likelihood methodology via Mega7 software, phylogenetic studies were performed, and BLAST searches of the NCBI database confirmed that all five isolates share nucleotide sequence similarity with the fungus *Botrytis cinerea*. The phylogenetic trees for all variants can be seen in Figure 3. The sequences, identified as SUB8901212 Seq1 MW485211, SUB8901212 Seq2 MW485212, SUB8901212 Seq3 MW485213, SUB8901212 Seq4 MW485214, and SUB8901212 Seq5 MW485215 can be found in the GenBank database. The use of molecular information and the construction of evolutionary trees play a crucial role in the identification process, as illustrated in Figure (4).

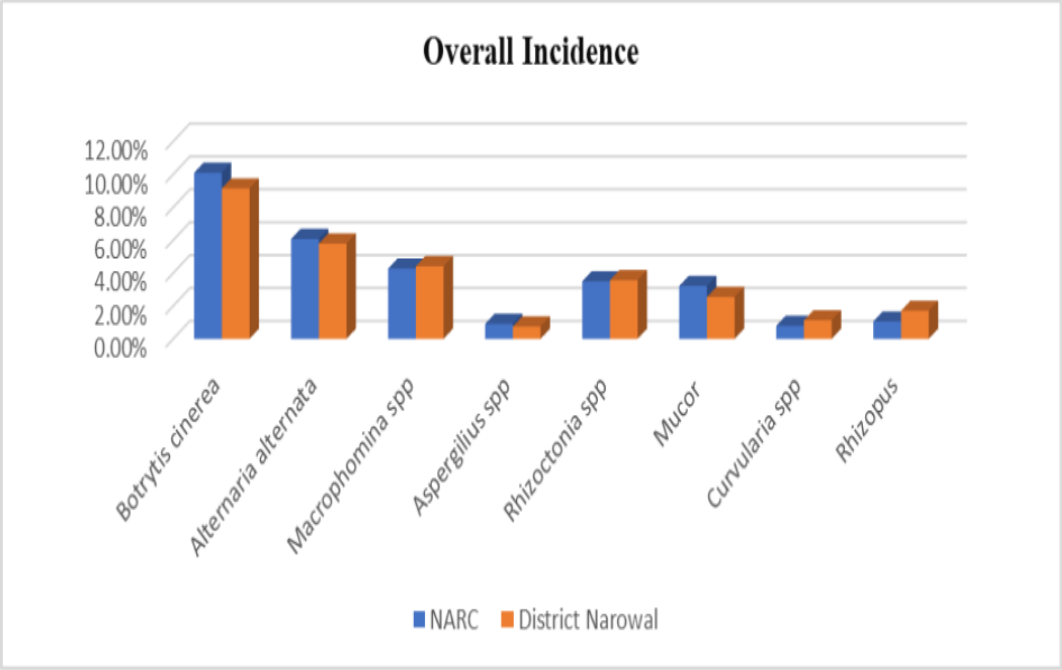

Figure 3: Overall incidence (%) of fungal species isolated from strawberry plants collected from NARC and Narowal districts.

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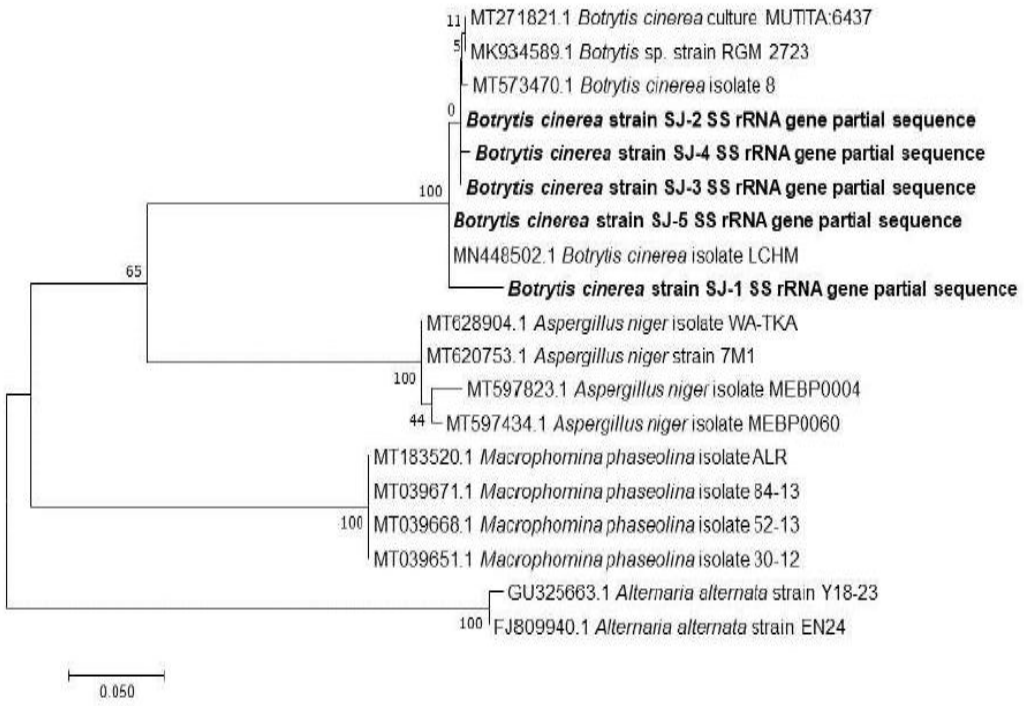

Figure 4: Phylogenetic Tree based on SS rRNA gene sequences of fungal isolates.

The in vitro study was performed to check the efficacy of different fungicides, antagonists, and plant extracts against *Botrytis cinerea* NARC and Narowal isolates on mycelial growth. Statistical analysis demonstrated that significant effect of different fungicides, antagonists, and plant extracts was observed against mycelia development of *Botrytis cinerea* at both NARC and Narowal sites. At both locations, all fungicide treatments showed a statistically significant reduction in fungal growth compared with the control group (Table 3), and Dicarboximide was most effective at both sites. Inhibitory activity was also displayed by Anilinoptrimidine, Benzimidazole, Pyrazole, Trizole and anilide. The difference in growth rates between control and treated samples is large, suggesting these treatments exerted a significant suppressive effect on the pathogen's growth.

While some chemicals showed better activity against Narowal isolates than NARC isolates, others even encouraged the growth of the pathogen (possible development of

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tolerance by fungi). The fungus was treated under laboratory conditions with seven compounds (six antagonists and one control). Using botanical extracts to establish ways to slow down the development of specific diseases is viewed as an ecologically sound practice. In this study, we investigated the effectiveness of botanical extracts for the inhibition of two strains of *Botrytis cinerea*. So, the competitive properties of six different botanical extracts and one placebo against several pathogenic microorganisms were tested. It has been determined that botanical extracts sourced from *Achillea millefolia*, *Allium sativum*, *Mentha spicata*, *Tagetes patula*, *Syzygium cumini*, and *Rosmarinus officinalis* display the highest effectiveness against NARC and Narowal strains.

Table 4. In vitro inhibitory effects of different fungicides on the mycelial growth of *Botrytis cinerea* isolates using the poisoned food technique.

Sours	df	Fung Narc	Fung Narowal	Anti narc	Anti narowal
Trt[1]	8	550.32	953.09	588.48	486.83
Error	18	0.535	0.669	1.84	1.44
Rep	4	2.33	0.271	3.67	4.88
total	30				

Discussion

Botrytis cinerea is one of the most destructive diseases of strawberries globally and, under favorable environmental conditions [2], it causes significant loss in yield and in fruit quality. Infected morphological identification was confirmed by pathogen screening and a pathogenicity test based on Koch's postulates [18]. Grey mold is a plant disease responsible for economically detrimental strawberry diseases and caused by the pathogenic fungus *Botrytis cinerea* [19]. Initially, we conducted a visual analysis component of the survey, focusing on identifying disease symptoms. The predominance of *B. cinerea* among the isolated fungi supports the notion that *B. cinerea* is the primary causative agent in strawberry gray mold under field environmental conditions within the areas surveyed for this study.

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The purified samples showed good results with respect to fungal growth development, as they were carefully studied under a microscope. Different sites in Narowal and NARC for the common grey mold disease (NARC had a higher frequency of this pathogen since it maintained higher humidity during the entire study [20]) were collecting samples in our research. The higher disease incidence reported in NARC may be correlated with prolonged leaf wetness, increased relative humidity, and temperatures that favor conidial germination, fungal infection, and sporulation of *B. cinerea*. Card [21]. Other reports backed up the findings. Several morphological and cultural characteristics of *B. cinerea* were also noticed between isolates in the course of this research and their global origins from outside of the indigenous strain were partly responsible for these differences.

Diverse studies based on cultural and morphologic characters have tentatively recognized *B. cinerea* [22]. Molecular confirmation using ITS-rDNA as a sequence region provides more taxonomic reliability, as its genomic adaptability may allow variability in morphologic characteristics between isolates from different environmental conditions; nevertheless, first identification based on colony morphology and microscopic habitat features remains helpful. To minimize the losses caused by this production disease, several in vitro experiments were performed to test the potential of various fungicidal medications, antagonists, and botanical extracts to treat this disease. There was a significant response to each of the six fungicides tested for fungal growth inhibition. The latter was the most efficient fungicide, followed closely by dicarboximide and benzimidazole.

Several studies have indicated that *B. cinerea* has developed resistance to the dicarboximide antibiotic. The phylogenetic analysis clustered the representative isolates with reference *B. cinerea* sequences available in GenBank, confirming the accuracy of the morphological identification and demonstrating limited genetic divergence among the analyzed isolates. Numerous other fungicide varieties, including benzimidazole, are available for consideration. Anilide, and triazole, among others [23-25]. Plant scientists are currently revisiting historical biological and agricultural methods as a result of the growing resistance to multiple fungicides and the escalating environmental concerns. One proposed solution is the utilization of pathogenic bacteria as agents of biological control to combat a variety of disorders that may impact the health of strawberry plants. Throughout our study, we tested six different antagonists to evaluate their effectiveness in inhibiting the

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pathogen in laboratory conditions. Among the antagonists examined, *Trichoderma spp*. demonstrated the highest efficacy against the pathogen, while *Bacillus subtilis, Penicillium spp., Burkholderia spp., and Colletotrichum spp*. also exhibited some ability to influence the disease. The conclusions drawn from our research align with previous studies conducted by other experts in the field [26-28]. In an experimental setting, a total of six distinct extracts were prepared and evaluated; each of these extracts exhibited significant efficacy against pathogens when compared to the control group. Among these extracts, *Allium sativum* extract demonstrated the highest effectiveness in inhibiting fungal growth, followed by extracts from *Mentha spicata*, *Tagetes patula*, *Syzygium cumini*, *Rosmarinus officinalis*, and *Achillea millefolium*.

Our findings extend the evidence obtained in the study carried out by [16,27–30]. that exalted *Allium sativum* extract as a more effective alternative for combating pathogenic fungi rather than standard pesticides. The prevention of grey mold disease in crops by fungi will require pre-harvest and post-harvest management strategies. The results of our study suggest that dicarboximide and benzimidazole can be promising and highly effective fungicides. The better activity shown by dicarboximide here may also be due to its interference in cell development and spore germination of fungal cells. Nonetheless, the development of resistant populations to this fungicide has been reported after repeated applications, hence the importance of resistance management studies. We are proposing an alternative to synthetic fungicides for the treatment of grey mold disease based on the use of *Allium sativum* and *Mentha spicata* extracts. Also, the use of biologicals such as *Trichoderma spp*. has been able to decrease the dependency on chemical treatments. This provides a platform for identifying resistant strawberry genotypes coupled with standard pathogenicity assays [31,32]. Moreover, detailed characterization of fungicide resistance phenotypes and screening new fungicides or alternative control agents (e.g., plant extracts or antagonistic microorganisms) are crucial to cope with the ample chemical insensitivity of the pathogen [33,34].

Additionally, the wide host range and phenotypic plasticity of *B. cinerea* requires ongoing identification of genetic diversity using multi-locus sequence typing to monitor for new strains emerging in agricultural systems [35,36]. Additionally, due to the high-diversity generalist nature of this pathosystem, disease management strategies using

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multiple approaches—cultural practices together with biological control agents and resistant cultivars—will remain an important mechanism for minimizing fruit yield losses across diverse crops [37,38]. The development of disease models based on near real-time climatic data would optimize the timing with which these interventions are applied and reduce unnecessary chemoprotective applications [39]. This huge difference in the rate of growth between both treatment groups and the negative controls may serve as definitive evidence that those treatments stopped or slowed the advancement of disease. Prolonged use of large quantities of pesticides has challenged sustainability, and molecular diagnostic tools alongside sustainable management strategies like antagonistic microorganisms can reduce pesticide residues in fields combined with quality maintenance of fruits [40,41]. Future studies should emphasize fast molecular diagnostic tools, such as TaqMan qPCR assays, that can be used to detect resistance-associated mutations in field populations [42]. This type of diagnostics focused on the IGS domain and/or genes associated with resistance to SDHI and benzimidazole fungicides would enable real-time monitoring of pathogen dynamics [43,44].

Conclusion:

The current study supported that Botrytis cinerea is the most common species causing gray mold disease of strawberry in nine production areas. Complementary evidence supporting accurate pathogen identification was obtained from morphological observations, pathogenicity testing and ITS–rDNA sequence analysis, but phylogenetic analysis confirmed that the representative isolates were closely related to reference *B. cinerea* sequences present in GenBank. The in vitro evaluation of different disease management strategies showed notable differences in the efficacy of the treatment tested. Of the fungicides evaluated, dicarboximide was most effective in promoting pathogen inhibition, while *Trichoderma spp*. was a more effective biological antagonist. Furthermore, while *Allium sativum* extract exhibited the strongest antifungal activity of all botanical extracts tested, it could be a promising green alternative or auxiliary ingredient for gray mold management.

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The results demonstrate that accurate molecular identification in combination with the comparative evaluation of chemical, biological and botanical control provides a promising foundation to develop holistic management strategies against strawberry gray mold. Future research needs include refining these treatments in both greenhouse and field settings, as well as assessing their long-term reliability as part of combined disease management regimes.

REFERENCES:

1. Prasannath, K.; Galea, V.J.; Akinsanmi, O.A. (2023). Diversity and pathogenicity of species of *Botrytis*, *Cladosporium*, *Neopestalotiopsis* and *Pestalotiopsis* causing flower diseases of macadamia in Australia. *Plant Pathol.* **2023**, *72*, 881–899.
2. Brauna-Morževska, E.; Stoddard, F.L.; Bankina, B.; Kaņeps, J.; Bimšteine, G.; Petrova, I.; Fridmanis, D. (2023). Evaluation of pathogenicity of *Botrytis* species isolated from different legumes. *Front. Plant Sci.* **2023**, *14*, 1069126.
3. Moparthi, S.; Parikh, L.P.; Gunnink Troth, E.E.; Burrows, M.E. (2023). Identification and prevalence of seedborne *Botrytis* spp. in dry pea, lentil, and chickpea in Montana. *Plant Dis.* **2023**, *107*, 382392.
4. Nielsen, K.A.; Skårn, M.N.; Strømeng, G.M.; Brurberg, M.B.; Stensvand, A. (2022). Pervasive fungicide resistance in *Botrytis* from strawberry in Norway: Identification of the grey mould pathogen and mutations. *Plant Pathol.* **2022**, *71*, 1392–1403.
5. Maia, J.N.; Beger, G.; Pereira, W.V.; De Mio, L.L.M.; Duarte, H.D.S.S. (2021). Gray mold in strawberries in the Paraná state of Brazil is caused by *Botrytis cinerea* and its isolates exhibit multiple-fungicide resistance. *Crop Prot.* **2021**, *140*, 105415.
6. Kulkarni, S.J. (2022). An insight into research and investigations of gray mold focused on *Botrytis cinerea*. In *Driving Factors for Venture Creation and Success in Agricultural Entrepreneurship*; Arafat, M.Y., Saleem, I., Ali, J., Khan, A., Balhareth, H.H., Eds.; IGI Global: Hershey, PA, USA, 2022; pp. 273–289.
7. Backmann, L., Schmidtmann, K., Wegmann-Herr, P., Jürgens, A. and Scharfenberger-Schmeer, M. (2024). Molecular biological methods to assess different Botrytis cinerea strains on grapes. Microbiology Research, 15(2), 567-581. https://doi.org/10.3390/microbiolres150 20037.

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8. Gaber, M.A., Wagih, E.E., Shehata, M.R., Fahmy, M.M., and Abdel Wahab, H.A. (2020). Detection and characterization of Botrytis cinerea isolates from vegetable crops in Egypt. International Journal of Phytopathology, 8(3), 77-85. https://journals.esciencepress.net/index. php/phytopath/article/view/2945.
9. Tuite, J. (1969) Plant Pathological Methods: Fungi and Bacteria. Burgess Publishing Company, 239 p.
10. Park, D., Shafer, O. T., Shepherd, S. P., Suh, H., Trigg, J. S., Taghert, P. H. (2008): The Drosophila basic helix - loop - helix protein DIMMED directly activates PHM, a gene encoding a neuropeptide - amidating enzyme. Molecular and Cellular Biology 28 (1): 410-421.
11. Shih, M., Ambady, N., Richeson, J. A., Fujita, K., & Gray, H. M. (2002). Stereotype performance boosts: The impact of self-relevance and the manner of stereotype activation. Journal of Personality and Social Psychology, 83, 638–647
12. Möller, E., Bahnweg , G., Sandermann, H. Geiger, H. (1992): A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. - Nucleic Acids Research 20 (22): 6115.
13. Sanger, F., Air, G. M., Barrell, B. G., Brown, N. L., Coulson, A.R., Fiddes, J. C., Hutchison, C. A., Slocombe, P. M. & Smith, M. (1977). Nature 265,687-695
14. Kumar, S., Stecher, G., Tamura, K. (2016): MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. - Molecular Biology and Evolution 33(7): 1870 1874 .
15. Fillinger, S. and Elad, Y. (Eds.). (2016). *Botrytis-the fungus, the pathogen and its management in agricultural systems* (Vol. **486**). Cham, Switzerland: Springer International Publishing. https://link.springer.com/content/pdf/10.1007/978-3-319-23371 -0.pdf.
16. Perez, C., Pauli, M. and Bazerque, P. (1990). An Antibiotic Assay by Agar Well Diffusion Method. Acta Biologiae et Medicinae Experimentalis, 15, 113-115.
17. Steel, R.G.D., Torrie, J.H. and Dickey, D.A. (1997) Principles and Procedures of Statistics: A Biometrical Approach. McGraw Hill Book Company, New York.

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18. Parducci, A., & Haugen, R. (1967). The frequency principle for com- parative judgments. Perception & Psychophysics, 2, 81-82

19. Petrasch, S. Knapp. S. J. Van Kan, J. A., Blanco - Ulate, B. (2019): Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen

20. Hassan, I., Mehmood, N., Riaz, A., Naz, F. (2019): Geographical distribution and morpho - molecular characterization of pre - harvest gray mold of strawberry in Punjab, Pakistan. - Mycopath 15 (2).

21. Card, S. D. (2005). Biological control of Botrytis cinerea in lettuce & strawberry crops. - A thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy at Lincoln University, Canterbury, New Zealand

22. Fernández - Ortuño, D., Li, X., Wang. F., Schnabel, G. (2012): First report of gray mold of strawberry caused by Botrytis caroliniana in North Carolina. - Plant Disease 96 (6): 914

23. Maia, J. N., Beger, G., Pereira, W. V., De Mio, L. L. M., Duarte, H. d. S. S. (2021): Gray mold in strawberries in the Paraná state of Brazil is caused by Botrytis cinerea and its isolates exhibit multiple - fungicide resistance. - Crop Protection 140: 105415.

24. Chen, X., Wang, Y., Gao, Y., Gao, T., Zhang, D. (2019): Inhibitory abilities of Bacillus isolates and their culture filtrates against the gray mold caused by Botrytis cinerea on postharvest fruit. - The Plant Pathology Journal 35 (5): 425.

25. Chen, X., Wang, Y., Gao, Y., Gao, T., Zhang, D. (2019): Inhibitory abilities of Bacillus isolates and their culture filtrates against the gray mold caused by Botrytis cinerea on postharvest fruit. - The Plant Pathology Journal 35 (5): 425.

26. Williamson, B., Tudzynski, B., Tudzynski, P. and Van Kan, J.A.L. 2007. *Botrytis cinerea*: The cause of grey mould disease. *Molecular Plant Pathology*, **8**(5), 561–580. https://doi.org/10.11 11 /j.1364-3703.2007.00417. x.

27. Staats, M., van Baarlen, P. and van Kan, J.A.L. 2005. Molecular phylogeny of the plant pathogenic genus *Botrytis* and the evolution of host specificity. *Molecular Biology and Evolution*, **22**(2), 333–346. https://doi.org/10.1093/molbev/ msi020.

28. Elad, Y., Williamson, B., Tudzynski, P. and Delen, N. (Eds.). 2007. *Botrytis: biology, pathology and control* (pp. 1-8). Sprin. https://link.springer.com/content/pdf/10.1007/978-1-4020-2626-3.pdf.

Published by DAC Democratic Arabic Center
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29. Basu, A., Nguyen, A., Betts, N. M., Lyons, T. J. (2014): Strawberry as a functional food: an evidence - based review. - Critical Reviews in Food Science and Nutrition 54 (6): 790 – 806.

30. Bertetti, D., Garibaldi, A., Gullino, M. (2008): Resistance of Botrytis cinerea to fungicides in Italian vineyards. - Communications in Agricultural and Applied Biological Sciences 73 (2): 273-282.

31. A R Joseph. Improved Management Strategies for Botrytis Fruit Rot, Macrophomina Root Rot, and Verticillium Wilt in California Strawberry Production. DigitalCommons - CalPoly (California State Polytechnic University), https://digitalcommons.calpoly.edu/theses/3144 (2025, accessed February 2026).

32. Jameel SM, Rehman A, Rajput N, et al. MOLECULAR CHARACTERIZATION OF THE PATHOGEN CAUSING GREY MOLD IN STRAWBERRY AND ITS MANAGEMENT. Applied Ecology and Environmental Research 2022; 20: 465–478.

33. Naegele RP, Abdelsamad N, DeLong J, et al. Fungicide Resistance and Host Influence on Population Structure in Botrytis spp. from Specialty Crops in California. Phytopathology 2022; 112: 2549–2559.

34. Radi M, Lahlali R. Management Strategies of Grey Mould Disease in Berries. Plant Health Cases. Epub ahead of print 24 March 2025. DOI: 10.1079/planthealthcases.2025.0010.

35. Abbey J, Alzohairy SA, Neugebauer KA, et al. Fungicide resistance in Botrytis cinerea and identification of Botrytis species associated with blueberry in Michigan. Frontiers in Microbiology 2024; 15: 1425392–1425392.

36. El-Beltagi HS, Abbas E, Almutairi HH, et al. Controlling Botrytis gray mold in strawberry fruit by bioactive protein isolated from kidney bean. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 2024; 52: 13783–13783.

37. Singh R, Caseys C, Kliebenstein DJ. Genetic and molecular landscapes of the generalist phytopathogen Botrytis cinerea. Molecular Plant Pathology; 25. Epub ahead of print 1 December 2023. DOI: 10.1111/mpp.13404.

38. Iqbal M, Flöhr A, Andréasson E, et al. Breeding for Integrated Pest Management (B-IPM): a new concept simultaneously optimising plant resistance and biocontrol. Frontiers in Plant Science 2025; 16: 1659069–1659069.

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39. Rehman A, Ali F, Hameed A, et al. Management Strategies for Botrytis Rot in Strawberry. In: IntechOpen eBooks. IntechOpen. Epub ahead of print 8 November 2023. DOI: 10.5772/intechopen.1002225.

40. Capriotti L, Molesini B, Pandolfini T, et al. RNA interference-based strategies to control Botrytis cinerea infection in cultivated strawberry. Plant Cell Reports 2024; 43: 201–201.

41. Šavli K, ŽUNIČ JZ, Celar F. Non-chemical control options against grey mould (Botrytis cinerea) on strawberry (Fragaria x anannasa). Journal of Central European Agriculture 2025; 26: 1015–1027.

42. Kim G, Son D, Choi S-G, et al. Monitoring of Benzimidazole Resistance in Botrytis cinerea Isolates from Strawberry in Korea and Development of Detection Method for Benzimidazole Resistance. The Plant Pathology Journal 2023; 39: 614–624.

43. Neugebauer KA, Mattupalli C, Hu M, et al. Managing fruit rot diseases of Vaccinium corymbosum. Frontiers in Plant Science 2024; 15: 1428769–1428769.

44. Keldibekova M, Knyazev SD. Genes and Loci Controlling the Resistance of Strawberry (F. ananassa Duch.) to Pathogens. Indian Journal of Agricultural Research. Epub ahead of print 17 November 2023. DOI: 10.18805/ijare.af-808.

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