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# Characterization and fungicide sensitivity of fungi causing postharvest deterioration in *Allium sativum*, Nuevo León, Mexico

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## ABSTRACT

**Background/Objective.** Garlic (*Allium sativum*) is a crop of economic relevance in Mexico. Nuevo León stands out in production; however, in the municipality of Aramberri, post-harvest losses have been reported due to diseases of unknown etiology. The objective of this work was to identify the fungi associated with the postharvest deterioration of *A. sativum* bulbs in Aramberri, Nuevo León, Mexico and to evaluate their *in vitro* sensitivity to fungicides.

**Materials and Methods.** From bulbs with evidence of deterioration and necrosis, fungi were isolated in PDA medium. Four isolates were identified by morphological analysis and one isolate from each morphological species was identified by molecular analysis. The pathogenicity of the four isolates on symptom-free bulbil was evaluated. In addition, *in vitro* susceptibility tests of the isolates to protective and systemic fungicides were performed. Fungicides were evaluated at three concentrations and mycelial growth reduction (MGR) and conidial germination inhibition (CGI) was estimated.

**Results.** The fungi *Alternaria embellisia* and *Penicillium allii* were identified in association with *A. sativum* bulbs with postharvest deterioration. *P. allii* showed the ability to develop internal infections from wounds; *A. embellisia* only showed growth on wounds. There were significant differences ($p < 0.0001$) in the effectiveness of fungicides on the two species. Propiconazole and copper hydroxide inhibited 100% MGR and CGI in both fungi, at all doses evaluated.

**Conclusion**. *P. allii* is first reported as a causative agent of green garlic rot in Mexico. This study will serve as a basis for choosing control strategies and will contribute significantly to reducing economic losses in garlic production in this region.

**Palabras clave:** Carbendazim, mycelial inhibition, germination inhibition, propiconazole, tebuconazole.

## INTRODUCTION

Garlic (*Allium sativum*) is a plant from the Amaryllidaceae family and an economically significant crop, valued for its medicinal and culinary properties. In 2023, global *A. sativum* production reached 28.6 million tons, with an average yield of 17 t $ha^{-1}$ (FAOSTAT, 2024). This plant produces secondary metabolites with antioxidant, anti-inflammatory, and hypolipidemic properties that contribute to human health (Ansary *et al*., 2020). Mexico plays a key role in global *A. sativum* production, with a cultivated area of 7,123 ha and an average yield of 12.6 t $ha^{-1}$ (SIAP, 2023). Nuevo León, ranks fifth place in garlic production, covering 289 ha with a yield of 11.9 t $ha^{-1}$. The municipality of Aramberri is the main producer, generating jobs and significant economic benefits (SEDAGRO, 2023).

Postharvest losses in garlic production occur mainly due to bulb rot (Sholberg and Conway, 2016). Several fungal pathogens have been identified as causal agents, including *Sclerotium cepivorum* (Burba, 2003); *Alternaria embellisia* (syn. *Embellisia allii*); *Botrytis porri*; *Aspergillus niger* (Dugan *et al.*, 2007; Khatoon *et al*., 2017); *A. ochraceus*; *Fusarium oxysporum*; *F. proliferatum*; *Penicillium hirsutum* (Dugan *et al*., 2007); *P. allii* (Valdez *et al.*, 2009); *F. solani*; and *Stemphylium vesicarium* (Gálvez and Palmero, 2021).

In Mexico, little information is available on fungi that attack *A. sativum* under storage conditions. However, some fungi have been reported in bulbs collected directly from the field, including *Fusarium verticillioides*, *F. acuminatum*, *F. solani*, and *F. oxysporum* (Ochoa-Fuentes *et al*., 2012). In stored bulbs, the presence of *P. hirsutum* Dierckx and *P. citrinum* (Hernández-Anguiano *et al.*, 2006), *A. embellisia* (Delgado-Ortiz *et al*., 2019), and the taxa *Ulocladium* sp., *Curvularia* sp., and *Verticillium* sp. (Velásquez-Valle *et al*., 2017) has been documented. These reports come from the states of Aguascalientes, Guanajuato, Coahuila, and Zacatecas, respectively. Some *A. sativum* diseases begin infecting plants during field development and continue progressing during storage. Additionally, certain pathogens can remain dormant and only develop in the postharvest phase (Chrétien *et al*., 2020). Effective disease management requires an integrated control system in which selecting and applying efficient phytosanitary products can help reduce the incidence of postharvest diseases.

In Nuevo León, symptoms of bulb rot have been observed under storage conditions in various plots in La Ascensión, Aramberri. This disease was first detected in 2017, with symptoms and signs including bulb cankers, cataphyll spots, black hyphae, and green mold. However, the causal agent(s) responsible for these infections in stored bulbs remain unidentified. Therefore, this study aimed to identify the fungi associated with postharvest deterioration of *A. sativum* bulbs in Aramberri, Nuevo León, Mexico, and to evaluate their *in vitro* sensitivity to fungicides. Understanding the causal agents and fungicide efficacy will aid in decision-making for postharvest disease control in *A. sativum*.

## MATERIALS AND METHODS

**Sampling and isolation of fungi.** In January 2022, five samples of infested garlic bulbs of the "Don Fermín" variety were collected from four storage facilities owned by producers in La Ascensión, Aramberri, Nuevo León, Mexico. The collected bulbs showed visible signs of deterioration and necrosis. The samples were placed in airtight

polyethylene bags and transported in coolers to the Phytopathology Laboratory at the Faculty of Agronomy, Autonomous University of Nuevo León, for processing.

Five infected bulbs were individually washed with sterile distilled water (SDW) and disinfected by immersion in 70% ethanol and 1% sodium hypochlorite for 1 min. This was followed by two additional rinses with SDW, each lasting two minutes. Five symptomatic tissue fragments were excised from each bulb and placed onto potato dextrose agar (PDA) medium. The Petri dishes containing the inoculated fragments were incubated at 25 ± 1 °C for seven days in darkness. A fragment of the PDA medium containing fungal mycelium was then transferred to a new Petri dish with PDA medium. From these polysporic isolates, monospore isolates were obtained.

To achieve this, a conidial suspension was prepared at a concentration of $1 \times 10^3$ conidia $mL^{-1}$, and 50 µL of the suspension was placed onto PDA plates. The plates were incubated at 25 °C and observed starting at 12 hours to identify germinated conidia isolated from others. The agar section containing a single germinated conidium was excised and transferred to a new PDA plate to develop the monospore isolate, following the procedures outlined by SENASICA (2016).

**Morphological identification**. Morphological identification at the genus and species level was conducted based on colony characteristics and micromorphology, following the criteria described by Vincent and Pitt (1989), Valdez *et al*. (2006), Lee *et al*. (2002), and Woudenberg *et al*. (2013). For this purpose, monospore isolates grown for seven days on PDA medium were used. Macroscopic characteristics, including colony size, shape, and conidial production, were evaluated.

Microscopic structures were obtained by inoculating a spore suspension onto PDA fragments, placing a sterile coverslip over the medium, and incubating the samples in a humid chamber. The morphology of conidiophores, phialides, conidia, and chlamydospores was observed using a ZEISS Scope.A1 microscope at 40× magnification. Photodocumentation of microscopic structures and micrometry were performed using ZEN lite® software.

**Molecular identification**. For each species identified morphologically, a single isolate was selected for molecular identification. DNA extraction was performed using the CTAB method from seven-day-old monospore cultures. DNA concentration was estimated by spectrophotometry (NanoDrop 2000, Thermo Scientific®). Two genomic regions were amplified by PCR: the ITS1-5.8S-ITS2 region of nuclear rDNA (ITS) for the morphologically identified species *A. embellisia,* and a partial sequence of the *β-tubulin* (B-TUB) gene for *P. allii*.

The ITS region was amplified using the primers ITS5 (5'-GCA AGT AAA AGT CGT AAC AAG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). Partial amplification of the *β-tubulin* gene was carried out using the primers Bt2a (5´-GGT AAC CAA ATC GGT GCT GCT TTC-3') and Bt2b (5'-ACC CTC AGT GTA GTG ACC CTT GGC-3'). PCR conditions for both genes included an initial denaturation at 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 90 s, with a final extension at 72 °C for 4 min. Amplifications were performed in 25 µL reaction volumes containing 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM of each primer (IDT), 1 unit of *Taq* DNA polymerase (Promega), and 20 ng of template DNA.

Amplicons were purified and sequenced by Psomagen (USA). The obtained sequences were compared against the non-redundant sequence database of the National Center for Biotechnology Information (NCBI). For phylogenetic analysis, sequences were aligned using MUSCLE (Edgar, 2004) implemented in MEGA-X (Kumar *et al.*, 2018). jModelTest v2 (Darriba *et al.*, 2012) was used to determine the most suitable nucleotide evolution model based on Akaike's information criterion. Phylogenetic analysis was performed with IQ-TREE v2 (Minh *et al*., 2020), which applies the Maximum Likelihood algorithm, using the CIPRES Science Gateway v3.3 (Miller *et al*., 2011). The Hasegawa-Kishino-Yano substitution model with a discrete Gamma distribution for rate heterogeneity (HKY + G) was used. Phylogenetic trees were visualized and edited with iTOL (Letunic *et al*., 2021).

**Pathogenicity tests.** Pathogenicity tests were conducted following the methodology of Gálvez and Palmero (2021), using two isolates per fungal species. Symptom-free garlic bulbs of the "Don Fermín" variety were selected. The outer scales were removed, and the cloves were disinfected by immersing them in 1% sodium hypochlorite for 1 min.

Each clove was wounded by removing a 5 mm diameter tissue fragment with a biopsy punch, creating a cavity approximately 4 mm deep. As inoculum, 5 mm diameter PDA agar plugs containing seven-day-old fungal mycelium were placed into the wounds. Control cloves were inoculated with PDA plugs without the pathogen. Five replicates were prepared for each fungal species.

After inoculation, the cloves were placed in a humid chamber and incubated at 25 °C for 14 days. Once lesions developed, tissue fragments were taken from the affected areas, rinsed with sterile distilled water (SDW), and placed onto PDA plates for incubation under the previously described conditions. The colonies that grew from these fragments were purified and morphologically identified. The successful re-isolation of the inoculated fungi confirmed compliance with Koch's postulates. The pathogenicity tests were performed twice to ensure consistency and reproducibility.

***In vitro* fungicide sensitivity test.** A single isolate from each identified species was tested for sensitivity to eight fungicides, four of which were systemic and four protectant (Table 1). Each fungicide was evaluated at three concentrations: 1X (the manufacturer's recommended dose), 0.5X, and 1.5X. Based on the recommended dose of the formulated product, the corresponding amount of active ingredient (g a.i. $L^{-1}$) was determined, considering the concentration in the commercial product. The chemicals were added to the PDA medium before pouring it into Petri dishes (Erhonyota *et al.*, 2023). The fungicides and their respective concentrations are shown in Table 1. The effect of the fungicides was evaluated on mycelial growth reduction (MGR) and conidial germination inhibition (CGI).

Seven-day-old fungal cultures grown on PDA medium were used to assess the effect of fungicides on mycelial growth reduction (MGR) and conidial germination inhibition (CGI) (Table 1). For MGR, 5 mm diameter plugs were extracted and individually placed at the center of Petri dishes containing PDA medium with the respective fungicide concentrations. The inoculated plates were incubated at 25 ± 0.5 °C, and MGR was recorded every 24 hours until the control (medium without fungicide) fully covered the plate. For CGI evaluation, a suspension of $5 \times 10^6$ conidia $mL^{-1}$ was prepared, and 30 µL were deposited onto PDA medium with the same treatments used for MGR evaluation,

with five replicates (Table 1). A sterile coverslip was placed over the inoculated spore suspension, and the plates were incubated at 25 ± 0.5 °C. Readings of 100 conidia per experimental unit were taken every 2 h until the control of each fungal species reached 90% germination. Conidia were considered germinated when the germ tube reached at least half the spore length. The inhibition percentage for MGR and CGI was calculated using the formulas IMGR = [(MGRtest - MGRtreat) / MGRtest] × 100, where IMGR = inhibition of mycelial growth (percentage), MGRtest = mycelial growth in the control treatment, and MGRtreat = mycelial growth in the fungicide treatment. Similarly, the inhibition percentage of CGI was calculated using the formula ICGI = [(CGItest - CGItreat) / CGItest] × 100, where ICGI = inhibition of conidial germination (percentage), CGItest = conidial germination in the control treatment, and CGItreat = conidial germination in the fungicide treatment.

**Table 1.** Systemic and protectant fungicides evaluated against *Alternaria embellisia* and *Penicillium allii*.

| Active Ingredient | Manufacturer | Chemical group | Mode of action | Dose (g AI $L^{-1}$)[z] | | |
|---|---|---|---|---|---|---|
| | | | | 0.5x | 1x[y] | 1.5x |
| Propiconazole | Syngenta | Triazole | Systemic | 0.625 | 1.25 | 1.875 |
| Thiabendazole | Syngenta | Benzimidazole | Systemic | 0.45 | 0.9 | 1.35 |
| Carbendazim | AgroLucava | Benzimidazole | Systemic | 0.5 | 1 | 1.5 |
| Azoxystrobin + Ciproconazole | Syngenta | Methoxyacrylates + Triazole | Systemic | 0.15/0.05 | 0.3/0.11 | 0.45/0.16 |
| Copper oxychloride | Cuprosa | Cupric | Protective | 6.525 | 13.05 | 19.575 |
| Captan | Mexfer | Phthalamides | Protective | 3.125 | 6.25 | 9.375 |
| Copper hydroxide | Cuprosa | Cupric | Protective | 3.75 | 7.5 | 11.25 |
| Tea tree oil | Syngenta | Monoterpenes | Protective | 0.555 | 1.11 | 1.665 |

[y] Recommended dose by the manufacturer.
[z] g AI = grams of active ingredient.

**Statistical analysis.** The effectiveness data for MGR and CGI were analyzed for each fungal species using a completely randomized experimental design. Before analysis, the data were transformed using the arcsine square root of the proportion. Treatment comparisons were then performed through an analysis of variance with a significance level of $p \leq 0.05$, followed by a post hoc Tukey test using the SAS® software (version 9.0; SAS Institute, Cary, N.C.).

## RESULTS AND DISCUSSION

**Observed symptoms.** The bulbs exhibited cataphylls with gray and black spots (Figure 1A), black mycelium between the cataphylls, and mold on the surface. Some bulbils showed superficial necrosis with a dry appearance and black sporulation, without progression into the interior of the bulbil. These symptoms were associated with *Alternaria* sp. Other bulbils displayed necrotic lesions with a yellowish border, sunken and watery in appearance, progressing into the interior of the bulbil. Additionally, they

showed mycelial growth and green sporulation. These symptoms were associated with *Penicillium* sp. (Figure 1B).

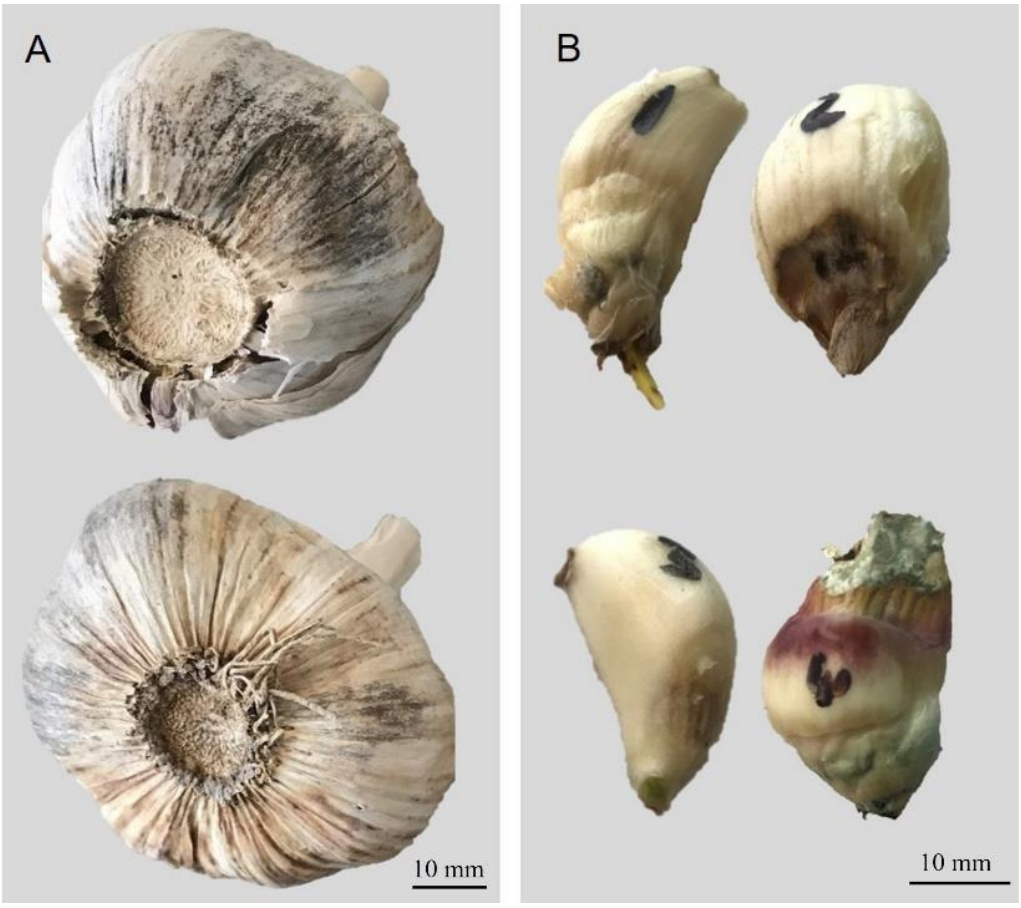


**Figure 1. A.** *Allium sativum* bulbs of the "Don Fermín" variety showing postharvest deterioration. **B.** *A. sativum* bulbils with cankers and green mold.

**Morphological Characterization.** Four isolates were obtained from *Allium sativum* bulbs showing postharvest deterioration (Figure 1): AL1D2B, AL1D3B, AL1D4B, and AL1D5B. Isolates AL1D2B and AL1D3B, obtained from superficial cankers, developed diffuse brownish-black colonies with both superficial and immersed mycelium (Figure 2A). Conidiophores were simple or branched, straight or flexible, measuring up to 83 µm in length and 5–10 µm in width (Figure 2B–E). Conidia were brown, solitary, smooth, oblong, with 4 to 6 transverse septa (commonly 4), measuring 19.0–34.6 µm in length and 9–12.9 µm in width (Figure 2F). Chlamydospores were terminal and intercalary, thick-walled, dark brown, and developed from hyphal cells (Figure 2G–H). These morphological characteristics are consistent with those described by Woudenberg *et al.* (2013) and Delgado-Ortiz et al. (2019) for *Alternaria embellisia* (syn. *Embellisia allii*).

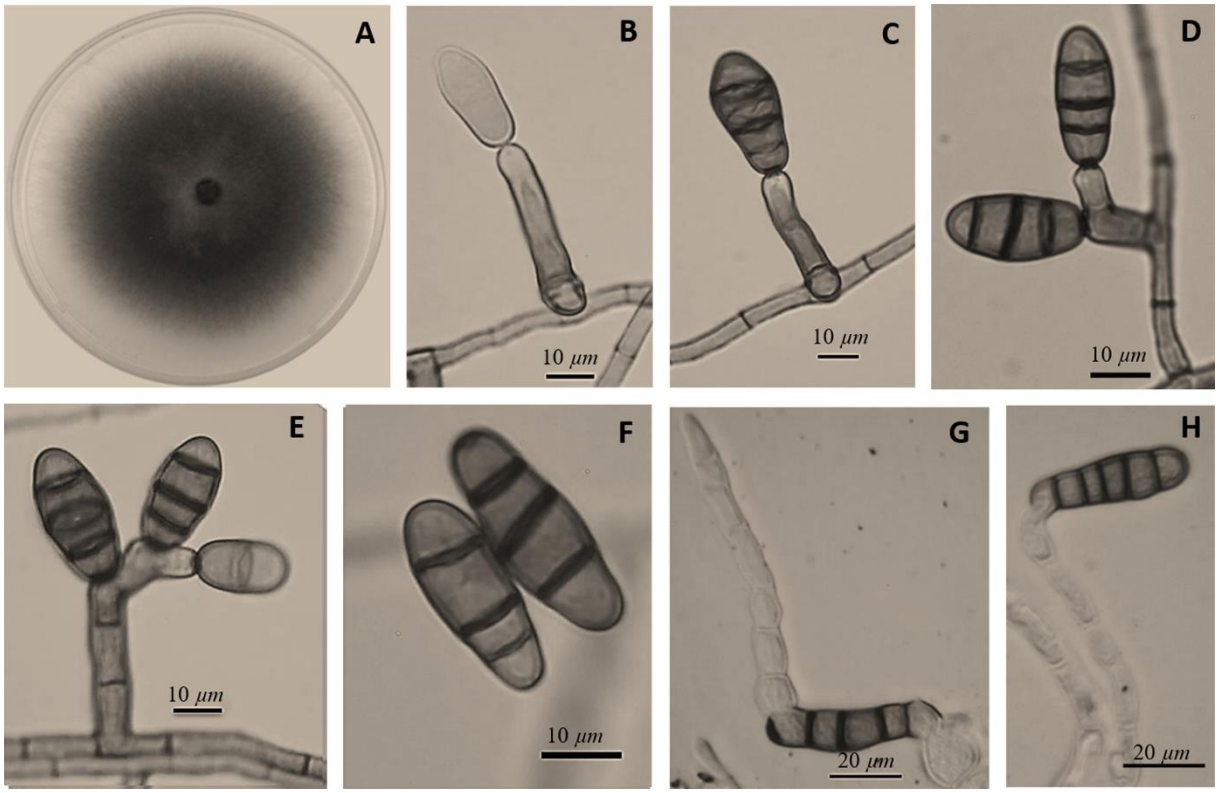


**Figure 2.** *Alternaria embellisia* (syn. *Embellisia allii*), isolates AL1D2B and AL1D3B. **A**. Seven-day-old colony growing on PDA medium at 25 °C**. B, C**. Simple conidiophore and solitary conidium. **D, E**. Branched conidiophore. **F**. Septate conidia. **G, H**. Chlamydospores.

The isolates AL1D4B and AL1D5B, obtained from lesions with mycelial growth and green sporulation, developed radially furrowed colonies that were moderately dense, with submerged margins and white mycelium, exhibiting moderate conidiogenesis and a dull green color (Figure 3A). Conidiophores with rough walls emerged from superficial hyphae in fascicles, with a short and robust basal cell measuring 96–155 × 3.4–5.1 µm (Figure 3B–D). Terminal penicilli had subterminal branches and recurrent branches with rough walls, typically with a single branch per penicillus, occasionally grouped into clusters of 2 to 3. Phialides were ampulliform, arranged in clusters of 2 to 4, measuring 6.4–11 × 2.7–3.3 µm. Conidia were spherical to subspherical, smooth-walled, and arranged in chains, with an average diameter of 3.6–4.9 µm (Figure 3E). These characteristics are consistent with those described by Vincent and Pitt (1989) and Valdez *et al.* (2006) for *Penicillium allii*.

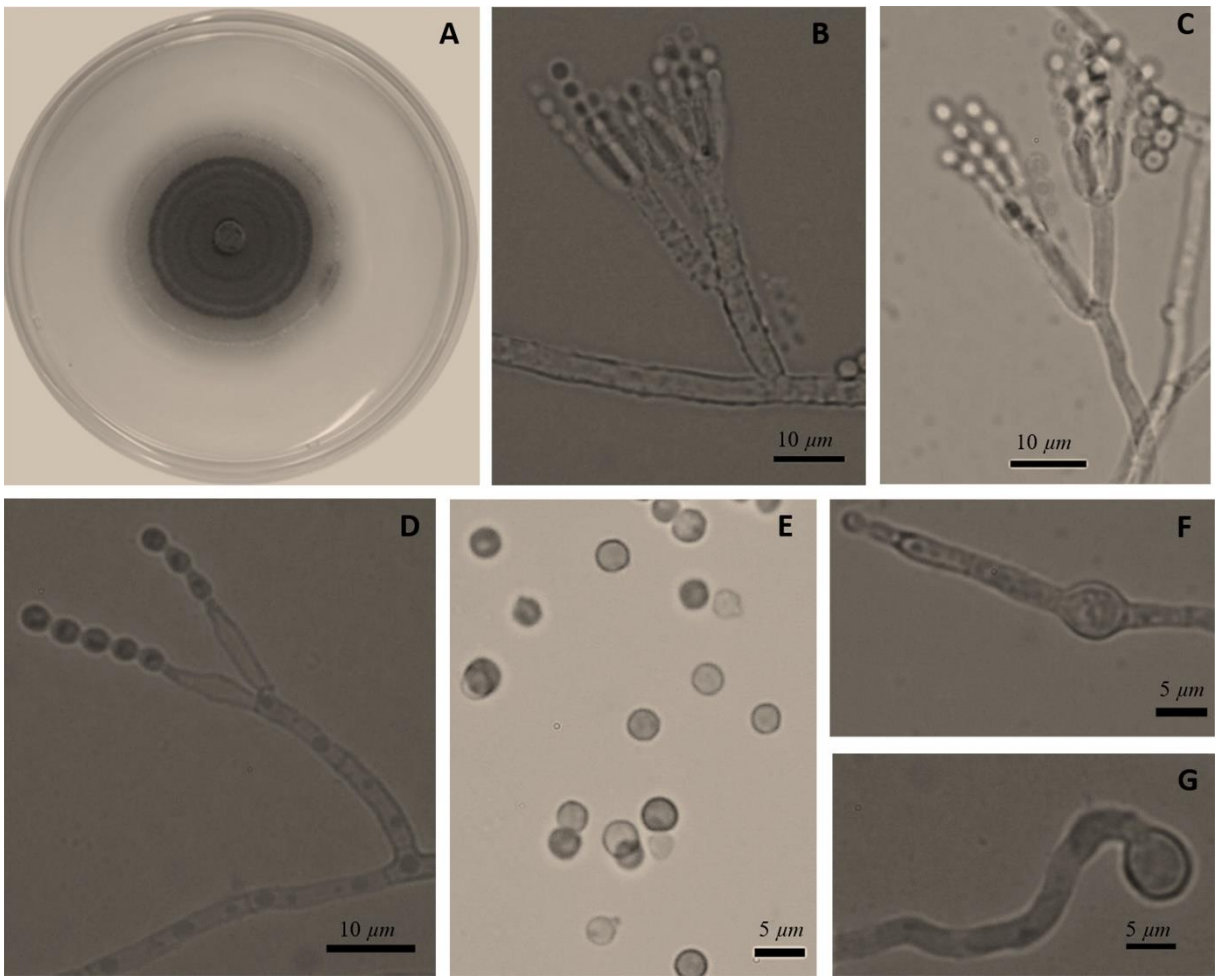


**Figure 3.** *Penicillium allii*, isolates AL1D4B and AL1D5B. **A**. Seven-day-old colony growing on PDA medium at 25 °C**. B**. Conidiophore showing stipe and metula with rough walls. **C**. Conidiophore. **D**. Conidiophore with phialides and conidiation forming chains. **E**. Conidia. **F, G**. Terminal and intercalary chlamydospores.

**Molecular Characterization.** The ITS region sequence of isolate AL1D2B (585 bp, GenBank accession number: PP869831) was 99.8% identical to the sequence reported for *A. embellisia* (MH899088) in Mexico (Delgado-Ortiz *et al*., 2019). Similarly, the β-tubulin region sequence of isolate AL1D4B (433 bp, GenBank accession number: PP920512) was 99.3% identical to the sequence reported for *P. allii* (MW244893) in Spain (Gálvez and Palmero, 2021). The phylogenetic tree constructed for *A. embellisia* (AL1D2B) showed bootstrap support of 100%, distinguishing our isolate from other *Alternaria* species and clustering it with *A. embellisia* (Figure 4A). Likewise, the phylogeny for *P. allii* (AL1D4B) exhibited bootstrap values of 100%, placing this isolate within *P. allii* (Figure 4B).

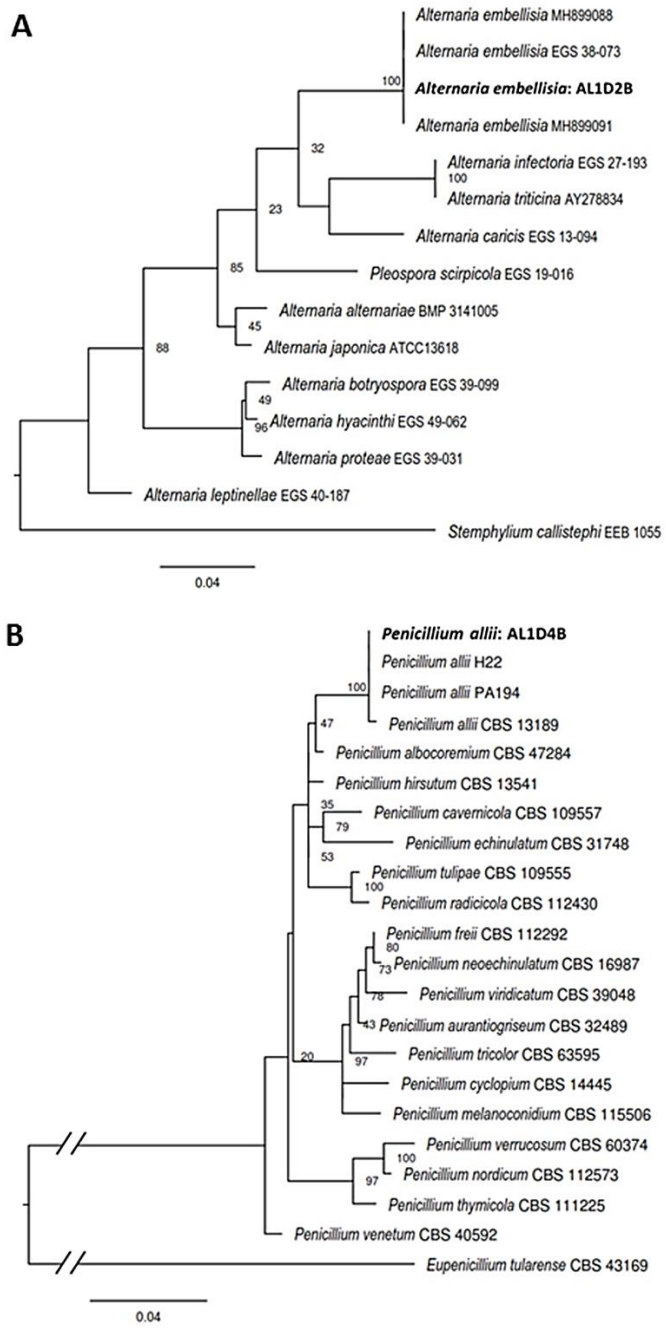


**Figure 4. A.** Maximum likelihood phylogenetic tree constructed from partial sequences of the internal transcribed spacer 1 and 2 regions within the 5.8S rDNA subunit for the isolation of *Alternaria embellisia* (GenBank accession number: PP869831). **B.** Maximum likelihood phylogenetic tree constructed from partial sequences of the β-tubulin gene for the isolation of *Penicillium allii* (GenBank accession number: PP920512); both obtained from garlic bulb samples with postharvest rot in Aramberri, Nuevo León. Data for other *Alternaria* strains and species were obtained from Pryor and Bigelow (2003), while data for other *Penicillium* strains and species were sourced from Samson et al. (2004). The numbers next to the nodes indicate bootstrap support values in percentage.

**Pathogenicity Tests.** The pathogenicity test conducted on *A. sativum* bulbils confirmed the pathogenicity of *P. allii* strains AL1D4B and AL1D5B, evaluated 14 days after

inoculation (dpi). All bulbils inoculated with *P. allii* developed sunken necrotic lesions with mycelial growth, green sporulation, and internal progression of the rot (Figure 4A). Similar results were reported by Gálvez and Palmero (2021), who observed sunken, watery lesions with green mycelial growth and internal lesion progression after inoculating garlic bulbils with *P. allii*.

Conversely, all bulbils inoculated with *A. embellisia* (AL1D2B and AL1D3B) exhibited mycelial growth and dark brown sporulation confined to the wound site created for inoculation at 14 dpi (Figure 4B). This observation aligns with the findings of Delgado-Ortiz *et al*. (2019) for *A. embellisia*, where *A. sativum* bulbs showed superficial growth, brown mycelium, and dark brown sporulation. Control bulbils showed no fungal infection (Figure 4C). The inoculated isolates that were re-isolated from infected tissue in all tested bulbils exhibited the same characteristics as the original isolates.

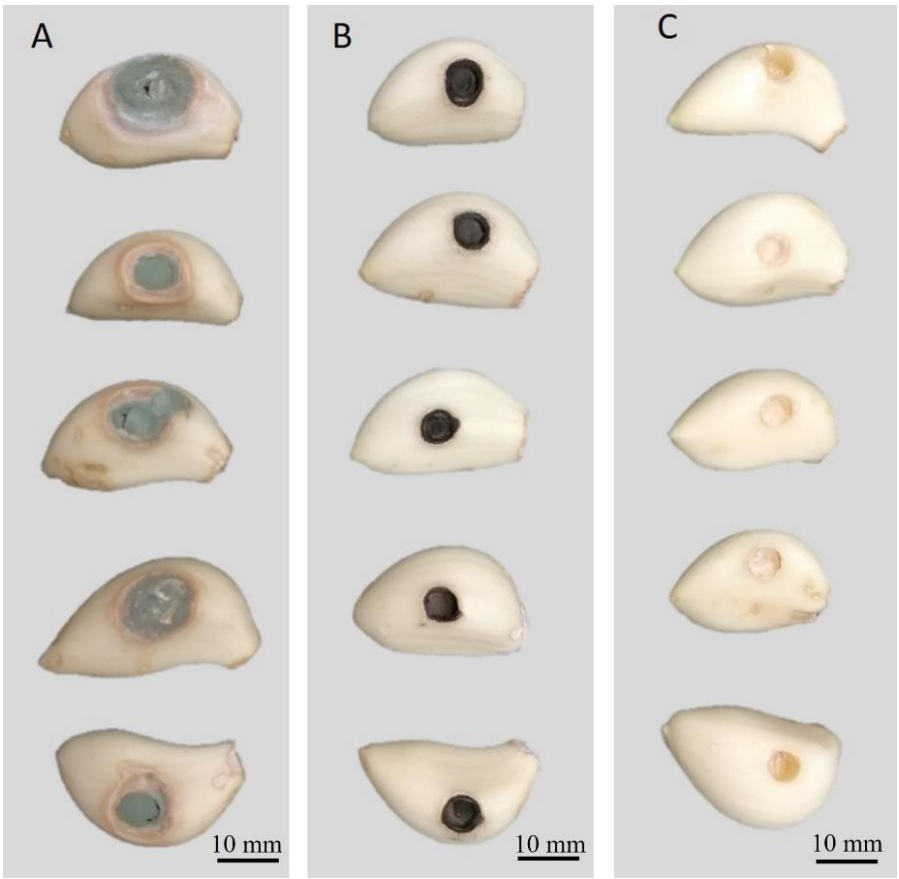


**Figure 5.** Pathogenicity test of (A) *Penicillium allii* (AL1D4B) and (B) *Alternaria embellisia* (AL1D2B) on *Allium sativum* bulbils, 14 days after inoculation. (C) Control. Each bulbil in the same column represents repetitions of the isolate.

Vincent and Pitt (1989) were the first to report and describe *P. allii* as the causal agent of *A. sativum* bulbil rot in Egypt. Subsequently, *P. allii* was identified as the cause of green garlic rot in the United States, China, Argentina, Egypt, and Spain (Dugan, 2007; Valdez *et al*., 2006; Moharam *et al*., 2013; Gálvez and Palmero, 2021). According to Gálvez and Palmero (2021), this fungus is one of the most virulent fungal pathogens

affecting garlic plants both in the field and during storage in Spain. These authors also reported that *P. allii* is more virulent in wounded bulbils and that this pathogen requires pre-existing wounds to develop. In Mexico, green garlic rot in stored bulbs has been attributed to *P. hirsutum* and *P. citrinum* (Hernández-Anguiano *et al*., 2006). To our knowledge, this is the first report of *P. allii* causing green rot in *A. sativum* bulbs under storage conditions in Mexico.

On the other hand, *A. embellisia* has been reported as the causal agent of cankers in stored bulbs and spots on the cataphylls of *A. sativum* bulbs and bulbils (Lee and Magan, 2010). This species was identified by Dugan *et al.* (2007) as a significant cause of losses in *A. sativum* bulbs during germplasm regeneration and storage for the National Plant Germplasm System in Pullman, Washington, USA. Additionally, this pathogen was reported as the causal agent of garlic bulb canker in Coahuila, Mexico (Delgado-Ortiz *et al.*, 2019). According to Lee *et al*. (2002), bulb canker caused by *A. embellisia* is considered a minor disease. However, due to the discoloration of symptoms, this disease can reduce the commercial value of *A. sativum*. Moreover, the presence of this fungus in bulbs can serve as a primary inoculum source and facilitate long-distance dispersal (Gálvez and Palmero, 2021).

***In Vitro* Sensitivity Tests to Fungicides.** *A. embellisia* and *P. allii* exhibited *in vitro* sensitivity to all evaluated fungicides, including both systemic and protectant types (Table 2). There were highly significant differences ($p < 0.0001$) in the effectiveness of the fungicides on the MGR and CGI of *A. embellisia*. In this case, the systemic fungicide propiconazole and the protectant fungicide copper hydroxide inhibited 100% of MGR and CGI at all three tested doses (Table 2). Similarly, the azoxystrobin + cyproconazole mixture inhibited 100% of MGR at all three doses but only inhibited CGI within a range of 40% to 85.9%, depending on the dose. Likewise, the fungicide captan inhibited 100% of CGI at all doses but only inhibited MGR within a range of 85% to 87% across the three evaluated doses. The fungicides with the lowest effectiveness against the MGR of *A. embellisia* were carbendazim, with a maximum effectiveness of 47% at the highest dose, and thiabendazole, with 76% effectiveness at the highest dose tested. The fungicides with the lowest effectiveness against CGI were thiabendazole (73% effectiveness) and tea tree oil (38.7% effectiveness) at the maximum evaluated dose (Table 2).

There were also highly significant differences ($p < 0.0001$) in the effectiveness of the fungicides on the MGR and CGI of *P. allii*. The systemic fungicide propiconazole and the protectant fungicide copper hydroxide inhibited 100% of MGR and CGI at all three tested doses (Table 2). Similarly, the protectant fungicide copper oxychloride inhibited 100% of MGR and CGI starting from the commercial dose (13.05 g.i.a. $L^{-1}$). Captan inhibited 100% of CGI at all evaluated doses but only achieved 100% MGR inhibition at the highest tested dose (9.3 g.i.a. $L^{-1}$). Likewise, carbendazim inhibited 100% of CGI only at 1.5 g.i.a. $L^{-1}$. The azoxystrobin + cyproconazole mixture inhibited 100% of CGI starting at 0.3/0.11 g.i.a. $L^{-1}$, while its effectiveness against MGR ranged from 83% to 89%.

**Table 2.** Effectiveness of systemic and protectant fungicides on mycelial growth and spore germination of *Alternaria embellisia* and *Penicillium allii in vitro*.

| Fungicide | Dose (g AI $L^{-1}$)[y] | *Alternaria embellisia* | | | | | | *Penicillium allii* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mycelial growth reduction (%)[z] | | | Germination inhibition (%)[z] | | | Mycelial growth reduction (%)[z] | | | Germination inhibition (%)[z] | | |
| Control | 0 | 0 | | | 0 | | | 0 | | | 0 | | |
| Propiconazole | 0.625 | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A |
| | 1.25 | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A |
| | 1.875 | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A |
| Thiabendazole | 0.45 | 71.7 | ±0.5 | C | 35.1 | ±7.4 | F | 38.9 | ±2.2 | GH | 50.0 | ±4.1 | F |
| | 0.9 | 75.1 | ±1.4 | C | 71.5 | ±4.1 | E | 51.5 | ±2.7 | F | 48.0 | ±2.4 | F |
| | 1.35 | 76.6 | ±1.2 | C | 73.1 | ±3.6 | E | 62.1 | ±1.2 | E | 48.5 | ±4.3 | F |
| Carbendazim | 0.5 | 34.6 | ±8.1 | E | 93.3 | ±0.6 | CB | 28.4 | ±3.5 | I | 92.5 | ±2.4 | CD |
| | 1.0 | 36.5 | ±4.3 | E | 97.4 | ±1.0 | B | 33.1 | ±4.1 | HI | 93.7 | ±3.1 | CB |
| | 1.5 | 47.5 | ±5.0 | D | 100 | ±0 | A | 33.4 | ±6.4 | IH | 100 | ±0 | A |
| Azoxystrobin + Ciproconazole | 0.15/0.05 | 100 | ±0 | A | 41.0 | ±8.2 | F | 83.4 | ±3.0 | D | 36.2 | ±4.5 | G |
| | 0.3/0.11 | 100 | ±0 | A | 88.2 | ±1.7 | DC | 85.6 | ±1.6 | D | 100 | ±0 | A |
| | 0.45/0.16 | 100 | ±0 | A | 85.9 | ±3.6 | D | 89.7 | ±0.6 | B | 100 | ±0 | A |
| Copper oxychloride | 6.525 | 71.9 | ±3.5 | C | 66.7 | ±6.7 | E | 100 | ±0 | A | 91.2 | ±0.5 | CD |
| | 13.05 | 84.9 | ±1.4 | B | 86.9 | ±2.3 | CD | 100 | ±0 | A | 100 | ±0 | A |
| | 19.575 | 100 | ±0 | A | 87.3 | ±1.4 | DC | 100 | ±0 | A | 100 | ±0 | A |
| Captan | 3.125 | 87.6 | ±2.3 | B | 100 | ±0 | A | 92.9 | ±1.0 | B | 100 | ±0 | A |
| | 6.25 | 85.6 | ±1.9 | B | 100 | ±0 | A | 90.3 | ±2.5 | B | 100 | ±0 | A |
| | 9.375 | 85.9 | ±1.3 | B | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A |
| Copper hydroxide | 3.75 | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A |
| | 7.5 | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A |
| | 1.665 | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A | 100 | ±0 | A |
| Tea tree oil | 0.55 | 45.3 | ±3.4 | D | 7.9 | ±0.5 | G | 15.4 | ±1.9 | J | 78.5 | ±1.9 | E |
| | 1.11 | 71.8 | ±2.6 | C | 20.7 | ±8.2 | G | 32.2 | ±4.0 | I | 88.7 | ±1.5 | D |
| | 1.665 | 100 | ±0 | A | 38.7 | ±8.3 | F | 44.9 | ±0.5 | FG | 96.0 | ±1.4 | B |

[y] **g AI** = grams of active ingredient.
[z] Means with the same letter within the same column are not statistically different (Tukey, $P < 0.05$).

The fungicides with the lowest effectiveness against the MGR of *P. allii* were thiabendazole, with a maximum effectiveness of 62% at the highest dose, carbendazim, with a maximum effectiveness of 33% at the highest dose, and tea tree oil, with a maximum effectiveness of 44.9% at the highest dose. The fungicide with the lowest effectiveness against CGI was thiabendazole, which showed 50% effectiveness at the highest dose tested, while tea tree oil showed CGI inhibition of 78% at the lowest dose and 96% at the highest dose evaluated.

According to Dugan *et al*. (2007), the use of fungicides can reduce bulb and bulbil infections in postharvest storage. However, it is important to consider the safety intervals of different active ingredients and the postharvest period. In this study, propiconazole and copper hydroxide demonstrated *in vitro* effectiveness against the MGR and CGI of *A. embellisia* at all tested doses.

Propiconazole is a systemic fungicide that inhibits methylation in ergosterol biosynthesis, causing disruption of the fungal cell membrane, which halts its growth (Marzi *et al*., 2022). Due to its systemic nature, this fungicide provides both preventive and curative protection, with persistence varying depending on the dose used and environmental conditions. Additionally, it can translocate into plant tissues, making it essential to consider pre-harvest intervals to ensure food safety. According to Gopinath *et al.* (2006), propiconazole can be metabolized in plants through the gradual degradation of its residues in plant tissues. Currently, no information is available regarding the use of propiconazole against *A. embellisia* in garlic crops. However, Vijaykumar *et al*. (2021) reported 100% effectiveness of tebuconazole, a fungicide from the same chemical group as propiconazole, against the mycelial growth of *A. porri*, a pathogen of *A. sativum* in India.

Copper hydroxide, on the other hand, is a protectant fungicide that offers preventive protection, acts through a multisite mechanism, has low toxicity, and presents a low risk of resistance development (Gaviria-Hernández *et al*., 2013). This fungicide primarily acts during the spore germination stage. At the cellular level, it reacts with sulfhydryl, hydroxyl, amino, and carboxyl groups, inactivating them and disrupting the respiratory chain (Gaviria-Hernández *et al.*, 2013). According to our literature review, no information is available regarding the use of copper hydroxide against *A. embellisia*. Other fungicides that have been effective in controlling *A. embellisia* in *A. sativum* bulbs stored in germplasm banks include fludioxonil (chemical group: phenylpyrroles) and thiophanate-methyl (chemical group: carbamates) (Dugan *et al.*, 2007).

Propiconazole and copper hydroxide also demonstrated 100% *in vitro* effectiveness against the MGR and CGI of *P. allii* at all tested doses. Similarly, no information is available regarding the use of propiconazole and copper hydroxide against *P. allii* in garlic cultivation. However, You *et al.* (2007) reported 100% effectiveness of tebuconazole against *P. hirsutum*, the causal agent of blue mold in *A. sativum* in Korea.

Additionally, the fungicides copper oxychloride and captan were effective against *P. allii*, inhibiting 100% of both MGR and CGI at doses of 13.05 g.i.a. $L^{-1}$ and 9.3 g.i.a. $L^{-1}$, respectively. Like copper hydroxide, copper oxychloride is a protectant fungicide that acts by releasing copper ions, operating through a multisite mechanism that provides preventive protection (Ackermann *et al*., 2000). Due to its multisite action, this compound has a low probability of resistance development in fungi (Fani *et al.*, 2021). Like other copper-based fungicides, copper oxychloride can persist on the surface of treated tissues, making it essential to follow usage recommendations and safety intervals to minimize copper residue accumulation in harvested products.

Captan, on the other hand, is a broad-spectrum, multisite fungicide that reacts with sulfhydryl enzymes, leading to the production of thiophosgene, a toxic substance for fungal cells. It interferes with the cellular respiration process in fungi, inhibiting spore germination and hindering mycelial growth and development. This fungicide has been used for over 50 years, with no reports of systemic toxicity (Ackermann *et al.*, 2000). To

our knowledge, no information is available regarding the use of copper oxychloride and captan against *P. allii*.

## CONCLUSIONS

This study identified *A. embellisia* and *P. allii* as fungi associated with the postharvest deterioration of *A. sativum* in Aramberri, Nuevo León, Mexico. Pathogenicity tests confirmed that *P. allii* causes green rot in garlic, demonstrating its ability to develop internal infections from wounds. This is the first report of *P. allii* as a causal agent of green rot in garlic in Mexico. *A. embellisia* was only able to grow on wounds, also contributing to the deterioration of *A. sativum* bulbs. The systemic fungicide propiconazole and the protectant fungicide copper hydroxide were the most effective, inhibiting 100% of both mycelial growth and conidial germination of *P. allii* and *A. embellisia in vitro* at all tested doses. Additionally, copper oxychloride and captan also exhibited high efficacy against *P. allii* and *A. embellisia*. The knowledge generated in this study provides a foundation for selecting control strategies and will contribute significantly to reducing economic losses in garlic production in this region. Further *in vivo* evaluations are necessary, applying fungicides in both preharvest and postharvest conditions.

### CONFLICTS OF INTEREST

The authors explicitly declare that there is no conflict of interest regarding this research.

## REFERENCES

Ackermann P, Margot P and Müller F. 2000. Fungicides, Agricultural. pp. 1-68. *In*: Müller F, Ackermann P, and Margot P. (eds.). Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH. New Jersey, USA. 9456p. https://doi.org/10.1002/14356007.a12_085

Ansary J, Forbes HTY, Gil E, Cianciosi D, Zhang J, Elexpuru ZM, Simal GJ, Giampieri F and Battino M. 2020. Potential health benefit of garlic based on human intervention studies: A brief overview. Antioxidants 9: 619. https://doi.org/10.3390/antiox9070619

Burba JL. 2003. Producción de ajo. Instituto Nacional de Tecnología Agropecuaria. Mendoza, Argentina. 43p. http://www.latranqueraweb.com.ar/web/archivos/menu/INTA_Produccion_de_ajo._.pdf

Chrétien PL, Laurent S, Bornard S, Troulet C, El Maâtaoui M and Leyronas C. 2020. Unraveling the infection process of garlic by *Fusarium proliferatum*, the causal agent of root rot. Phytopathologia Mediterranea 59: 285–293. https://doi.org/10.14601/Phyto-11103

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and high-performance computing. Nature methods 9: 772. https://doi.org/10.1038/nmeth.2109.

Delgado-Ortiz JC, Cerna-Chavez E, Ochoa-Fuentes YM and Beltrán-Beache M. 2019. First report of *Alternaria embellisia* (Syn. *Embellisia allii*) causing bulb canker or skin blotch on garlic in Mexico. Plant Disease 103: 1031. https://doi.org/10.1094/pdis-07-18-1171-pdn

Dugan FM, Hellier BC and Lupien SL. 2007. Pathogenic fungi in garlic seed cloves from the United States and China, and efficacy of fungicides against pathogens in garlic germplasm in Washington State. Journal of Phytopatholy 155: 437–445. https://doi.org/10.1111/j.1439-0434.2007.01255.x

Dugan FM. 2007. Diseases and disease management in seed garlic: Problems and prospects. The Americas Journal of Plant Science and Biotechnology 1: 47–51. http://www.globalsciencebooks.info/Online/GSBOnline/images/0706/AmJPSB_1(1)/AmJPSB_1(1)47-51o.pdf

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic acids research 32: 1792-1797. https://doi.org/10.1093/nar/gkh340

Erhonyota C, Edo GI and Onoharigho FO. 2023. Comparison of poison plate and agar well diffusion method determining the antifungal activity of protein fractions. Acta Ecologica Sinica, 43: 684-689. https://doi.org/10.1016/j.chnaes.2022.08.0062023

Fani SR, Azimi H and Probst C. 2021. Efficacy of copper oxychloride base fungicides to control cucumber downy mildew in greenhouse conditions in Iran. Journal of Crop Protection 10: 523-533 http://jcp.modares.ac.ir/article-3-47785-en.html

FAOSTAT, The statistics division of the Food and Agriculture Organization of the United Nations. 2024. Value of agricultural production. https://www.fao.org/faostat/en/#data/QV (consulta, enero 2025).

Gálvez L and Palmero D. 2021. Incidence and etiology of postharvest fungal diseases associated with bulb rot in garlic (*Alllium sativum*) in Spain. Foods 10: 1063. https://doi.org/10.3390/foods10051063

Gaviria-Hernández V, Patiño-Hoyos LF y Saldarriaga-Cardona A. 2013. Evaluación *in vitro* de fungicidas comerciales para el control de *Colletotrichum* spp., en mora de castilla. Ciencia y Tecnología Agropecuaria, 14 67-75. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0122-87062013000100008&lng=en&tlng=es.

Gopinath K, Radhakrishnan NV and Jayaraj J. 2006. Effect of propiconazole and difenoconazole on the control of anthracnose of chilli fruits caused by *Colletotrichum capsici*. Crop Protection 25: 1024-1031. https://doi.org/10.1016/j.cropro.2006.02.001

Hernández-Anguiano AM, Juárez-López G, Fucikovsky Zak L, Zavaleta-Mejía E y González Hernández VA. 2006. Impacto del almacenamiento en la brotación de bulbos de ajo y especies patogénicas de *Penicillium* y *Erwinia* asociadas. Revista Fitotecnia Mexicana 29: 283-290. https://doi.org/10.35196/rfm.2006.4.283-290

Khatoon A, Mohapatra A and Satapathy KB. 2017. Studies on fungi associated with stora garlic (*Allium sativum*). International Research Journal of Biological Sciences, 6: 19–24. https://www.isca.me/IJBS/Archive/v6/i1/4.ISCA-IRJBS-2016-148.pdf

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution 35: 1547. https://doi.org/10.1093/molbev/msy096

Lee HB and Magan N. 2010. The influence of environmental factors on growth and interactions between *Embellisia allii* and *Fusarium oxysporum* f. sp. *cepae* isolated from garlic. International Journal of Food Microbiology 138: 238–242. https://doi.org/10.1016/j.ijfoodmicro.2010.01.019

Lee HB, Kim CJ and Yu SH. 2002. First report of bulb canker of garlic caused by *Embellisia allii* in Korea. Mycobiology 30: 240–243. https://doi.org/10.4489/MYCO.2002.30.4.240

Letunic I, Khedkar S and Bork P. 2021. SMART: recent updates, new developments and status in 2020. Nucleic acids research 49: 458-460. https://doi.org/10.1093/nar/gkaa937

Marzi M, Farjam M, Kazeminejad Z, Shiroudi A, Kouhpayeh S and Zarenezhad E. 2022. A recent overview of 1,2,3-triazole-containing hybrids as novel nntifungal agents: focusing on synthesis, mechanism of action, and structure-activity relationship (SAR). Journal of Chemistry. 2022: 1-50. https://doi.org/10.1155/2022/7884316

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A and Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Molecular biology and evolution 37: 1530-1534. https://doi.org/10.1093/molbev/msaa015

Moharam MHA, Farrag ESH and Mohamed MDA. 2013. Pathogenic fungi in garlic seed cloves and first report of *Fusarium proliferatum* causing cloves rot of stored bulbs in upper Egypt. Archives of Phytopathology and Plant Protection 46: 2096–2103. https://doi.org/10.1080/03235408.2013.785122

Miller M, Pfeiffer WT and Schwartz T. 2011. The CIPRES science gateway: A community resource for phylogenetic analyses. Proceedings of the TeraGrid Conference 2011: 1-8. https://doi.org/10.1145/2016741.2016785

Ochoa-Fuentes YM, Cerna-Chavez E, Gallegos-Morales G, Landeros-Flores J, Delgado-Ortiz JC, Hernández-Camacho S y Olalde-Portugal V. 2012. Identificación de especies de *Fusarium* en semilla de ajo en Aguascalientes, México. Revista Mexicana de Micología 36: 27-32. https://doi.org/10.33885/sf.2012.3.1102

Pryor BM and Bigelow DM. 2003. Molecular characterization of *Embellisia* and *Nimbya* species and their relationship to *Alternaria*, *Ulocladium* and *Stemphylium*. Mycologia 95: 1141-1154. doi: 10.1080/15572536.2004.11833024.

Samson RA, Seifert KA, Kuijpers AF, Houbraken JAMP and Frisvad JC. 2004. Phylogenetic analysis of *Penicillium* subgenus *Penicillium* using partial β-tubulin sequences. Studies in Mycology 49: 175-200. https://www.studiesinmycology.org/sim/Sim49/Sim49Article2.pdf

SEDAGRO, Secretaría de Desarrollo Agropecuario del Estado de Nuevo León. 2023. Detonará NL la exportación de ajo del Sur del Estado. https://www.nl.gob.mx/boletines-comunicados-y-avisos/. (consulta, enero 2024).

SENASICA, Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria. 2016. Cultivos monospóricos de hongos entomopatógenos. https://www.gob.mx/cms/uploads/attachment/file/172904/Ficha_CB_27_Cultivos_monosp_ricos_de_hongos_entomopat_genos.pdf (consulta, noviembre 2023).

Sholberg PL and Conway WS. 2016. Postharvest pathology. Pp: 111–127. *In*: Gross KC, Wang CY and Saltveit M. (eds.). The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks. USDA-ARS Agriculture Handbook. United States. 790p.

SIAP, Servicio de Información Agroalimentaria y Pesquera. 2023. Producción Agricola. https://www.gob.mx/siap/acciones-y-programas/produccion-agricola-33119 (consulta, enero 2024).

Valdez JG, Makuch MA, Ordovini AF, Frisvad JC, Overy DP, Masuelli R and Piccolo RJ. 2009. Identification, pathogenicity and distribution of *Penicillium* spp. isolated from garlic in two regions in Argentina. Plant Pathology 58: 352–361. https://doi.org/10.1111/j.1365-3059.2008.01960.x

Valdez JG, Makuch MA, Ordovini AF, Masuelli RW, Overy DP and Piccolo RJ. 2006. First report of *Penicillium allii* as a field pathogen of garlic (*Allium sativum*). Plant Pathology 55: 583. https://doi.org/10.1111/j.1365-3059.2006.01411.x

Velásquez-Valle R, Macias-Valdez LM y Reveles-Hernández M. 2017. Patógenos comunes de semilla de ajo en Aguascalientes y Zacatecas, México. Revista mexicana de Ciencias Agrícolas 8: 1881-1885. https://doi.org/10.29312/remexca.v8i8.710

Vijaykumar KN, Kulkarni S, Patil PV, Kambrekar DN and Shashidhar TR. 2021. *In vitro* evaluation of new molecules of fungicides against purple blotch *Alternaria porri* (Ellis) Ciferri of garlic (*Allium sativum* L.). Pharma Innovation Journal 10: 1048-1054. https://www.thepharmajournal.com/archives/2021/vol10issue12/PartO/10-11-159-761.pdf

Vincent MA and Pitt JI. 1989. *Penicillium allii*, a new species from egyptian garlic. Mycologia 81: 300–303. https://doi.org/10.2307/3759715

You O, Lee Y, Jin Y, Kim J, Hwang S, Han S and Kim J. 2007. Antifungal activity of pesticides to control dry rot and blue mold during garlic storage. The Korean Journal of Pesticide Science 11: 331-338. https://koreascience.or.kr/article/JAKO200709906341813.pdf

Woudenberg JH, Groenewald JZ, Binder M and Crous PW. 2013. *Alternaria* redefined. Studies in Mycology 75:171-212. http://dx.doi.org/10.3114/sim0015